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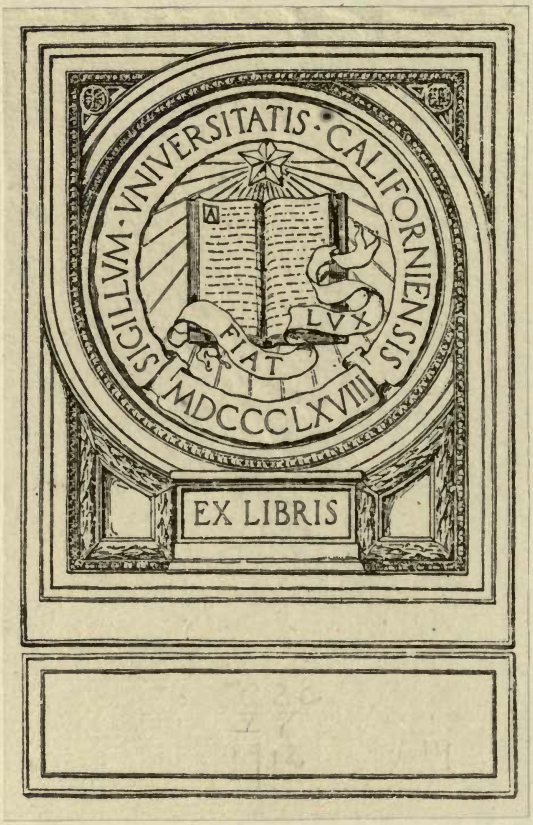
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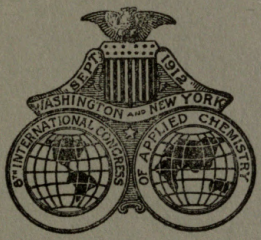
SECTION VIIId: BIOCHEMISTRY INCLUDING PHARMACOLOGY



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VOLUME XIX

SECTION VIII*d*: BIOCHEMISTRY INCLUDING PHARMACOLOGY

CONTENTS

THE IODINE CONTENT OF THE SMALL, MEDIUM AND LARGE THYROID GLANDS OF SHEEP,[1] BEEF AND HOGS

By T. B. Aldrich

(From the Research Laboratory of Parke, Davis & Co., Detroit, Mich.)

It is conceded by a majority, if not all writers on the subject of thyroid therapy, that the thyroid gland (or its preparations) to be physiologically active, must contain at least *some* iodine, furthermore that this iodine to be of the greatest value therapeutically, must be combined or associated with some protein or organic complex found in the gland. Presumably the iodine is the more important constituent, the two, however, associated or combined seem to give the best therapeutic results and to-day the efficiency of a thyroid preparation is generally measured, or should be by its iodine content. In fact for some time a number of pharmaceutical houses have been putting out thyroid preparations with a guaranteed percentage of iodine. Since then the iodine content of a thyroid preparation is a measure of its therapeutic efficiency, it is desirable to select if possible those glands which contain the most iodine, providing other factors are equal, and from those animals, whose thyroids contain the most of this constituent.

The following work was taken up with the object of determining the iodine content and thereby the therapeutic efficacy of some thyroid glands, especially the small, medium and large thyroid glands of sheep, beef and hogs by means of the method employed by Hunter (Journal of Biolog. Chemistry 1909-1910, VII, p. 321) which is very accurate and detects the presence of very small amounts of iodine, that are incapable of being detected by the older method of Baumann (J. Physiol. Chem. 1896, 21, p. 489; Ibid, 22, p. 1) which has been the method usually employed heretofore. (See Rigg's work J. of American Chem. Soc. 1910) XXXII, p. 692; Ibid, 1909 XXXI, p. 710).

[1]The iodine content of some mixed sheep thyroids was also determined.

The glands received came from the Chicago stock yards, were placed in Mason jars as soon as removed from the animals, subsequently placed in cold storage and shipped packed in ice. They were all received in the best possible condition. Six lots were obtained as follows:

(1) Mixed sheep thyroids (Lots A and B).
(2) Small, medium and large sheep thyroids (Lot C).
(3) " " " " " " (Lot D).
(4) " " " " beef " (Lot E).
(5) " " " " hog " (Lot F).

In lots A, B and C the glands were not counted.

After freeing from superfluous tissue and weighing, the glands were ground very fine, defatted and desiccated in the usual manner and eventually reduced to a very fine powder by passing through a 60 mesh sieve.

The following information obtained by one* of our staff from a packer, relative to thyroids, may be of interest at this point.

(1) Sheep thyroids are subject to great variation in size. The sex factor is not the determining factor for the size of the gland, nor has the condition of nutrition of the sheep any decisive bearing on the size of the gland. They run from the size of an almond to the size of a lemon.

(2) Steers' thyroids are larger than cows', this variation in size being a *constant* factor; size of gland varies again with the condition of the animal. Well-nourished cattle have larger thyroids than poorly fed ones.

(3) In hogs the thyroids vary little in size and present only slight variation in general appearance.

The thyroids of cattle are removed after head has been severed; same is true of hogs. Cattle thyroids are often cut, those of hogs not.

The following method of assaying the iodine, somewhat abbreviated was employed. (For details see Hunter, The Journal of Biological Chemistry 1909-1910, VII, p. 321).

Exactly one gram of the body was taken, placed in a nickel crucible (125 c.c.), 14 grm. of the following oxidation mixture added (106 parts Sodium carbonate, 75 parts Potassium Nitrate,

and 138 parts Potassium carbonate) and the two intimately mixed by means of a nickel spatula. Over this was dusted 4 gm. of the oxidation mixture. The nickel crucible was then heated over a flame until contents of the same was perfectly white. This more or less fused mixture was dissolved in water and brought into an Erlenmeyer flask (800 cc.). After cooling 35 cc. of Sodium Hypochlorite solution was added, and while holding the flask in a slanting position in cold water and agitating at the same time, 65 cc. of $42\frac{1}{2}\%$ phosphoric acid was added. The solution should, after the addition of the acid, be colored slightly yellow from the slight excess of chlorine liberated. The mixture was then boiled briskly, a funnel with a short stem being placed in neck of flask to avoid any loss. When all the free chlorin was expelled, recognized by holding filter paper moistened with starch solution containing Potassium iodine in the steam (blue color if present), the flask containing now about 70-80 cc. was allowed to cool and brought up to about 200 cc. by the addition of water. To this cold solution 10 cc. of a 1% Potassium Iodide solution was added, which causes the liberation of 6 times the amount of iodine originally in the product to be assayed, according to the following equation:

$$KIO_3 \cdot HIO_3 + 10\ KI + 11\ HCl = 11\ KCl + 6\ H_2O + 12\ I$$

This liberated Iodine is immediately titrated with a standard solution of Sodium thio sulphate solution approximately N/200, a few drops of starch solution being added toward the end of the reaction.

The number of Cc. of Sodium thio sulphate used multiplied by the iodine factor then divided by 6 gives the amount of iodine in the original sample.

A blank test using casein, or some other body free from iodine was made to insure the absence of iodine in the reagents, and the usual precautions employed in analytical methods observed.

The following table gives the number (except in the lots A, B and C), total weight of glands, average weight of glands and iodine content in each lot with average iodine content and percentage in each gland, where the number of glands is known.

SHEEP THYROIDS (LOTS A AND B)

	No. of Glands	Total Wt. of glands gms.	Av. Wt. of glands gms.	Iodine % (moist gland tissue)		Av. iodine in each moist gland mg.	%
(A)		2925 (MX)[1]		.032	.025		
(B)		5120 "		.022			

SHEEP THYROIDS (LOT C)

(1)		6000 (S)		.027			
(2)		5200 (M)		.018	.019		
(3)		5805 (L)		.01			

SHEEP THYROIDS (LOT D)

(1)	397 (S)	540	1.36	.044	0.028	.6	.04
(2)	192 (M)	650	3.38	.028		.9	.027
(3)	48 (L)	675	14.00	.015		2.1	.015

BEEF THYROIDS (LOT E)

(1)	98 (S)	620	6.32	.039	0.036	2.47	.04
(2)	53 (M)	405	7.64	.030		2.40	.031
(3)	34 (L)	425	12.20	.038		4.70	.038

HOG THYROIDS (LOT F)

(1)	108 (S)	725	6.7	.054	0.047	3.6	.05
(2)	70 (M)	765	10.9	.048		5.2	.047
(3)	40 (L)	735	18.37	.041		7.5	.04

[1]The letters (S) (M) (L) and (MX) stand for small, medium, large and mixed glands.

The average iodine content of the mixed sheep thyroid glands, Lots (A), (B), (C), and (D) where over 50 lbs. were employed, is about .025% while in some cases with selected glands 0.044% has been obtained and .027 where relatively large amounts (6000 gms.) were used.

The beef thyroids (Lot E) gave an average of .036% iodine, and in selected cases nearly .04%. The hog .047% average, in selected glands over .05% iodine.

It will be noted that the greatest variation in iodine content in the different sized glands of the same animals, is in that of the sheep where it varies from .01%-.027% in Lot C and from .015-.044 in Lot D. This variation being no doubt due to the greater prevalence of goiter in sheep.

Next to sheep the iodine content of the hogs' thyroids vary the most .041-.054% while in the beef we have the least variation .030-.39%.

The hogs' thyroids[1] contain the highest percentage of iodine, with the small sheep glands in one case higher, in the other lower than the mixed beef glands. Assuming the iodine content to be a measure of the therapeutic activity, the mixed thyroids of the hog are superior to the beef and the latter superior to sheep thyroids, small sheep thyroids being about equal to mixed beef thyroids.

Weight for weight the small glands of all the animals studied, nearly without exception contain the most iodine (excepting beef where the large and the small have nearly the same .038%).

In general the larger glands contain the most iodine and the ratio of the iodine content of the small, medium and large glands is approximately as follows:

In the sheep 2: 3: 7
" " beef 1: 1: 2
" " hog 3: 4: 6

The mixed glands arranged according to their iodine content stand about in the following ratio:

[1]It is interesting to note that Baumann found very little iodine in pigs' and hogs' thyroid glands; *very much* less than in beef and sheep.

Sheep	Beef	Hogs
5	7	9

From whatever standpoint we take we must conclude from the above that the employment of either hogs or beef thyroids for therapeutic purposes would be more rational than the employment of sheep glands, even if small selected sheep glands are employed, thus eliminating the goiterous glands.

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*It is a pleasure to thank Dr. Baeslack, of our staff, for looking after the collection of the glands and also for the information relative to the same.

BIOCHEMICAL AND TOXICOLOGICAL STUDIES ON PENICILLIUM STOLONIFERUM — THOM

By C. L. Alsberg and O. F. Black

United States Department of Agriculture, Washington, D. C.

Whether molds or the products of their growth have an injurious effect on animals is a question which has not yet been conclusively settled. The literature contains many records of alleged intoxications due to these fungi. Certain diseases of men and domesticated animals have been attributed to this cause. Though, obviously, the solution of this problem is urgent, few serious attempts have been made to identify chemically the alleged toxic substances. Chemical studies of this kind have been undertaken in the Poisonous Plant Laboratory of the Office of Drug Plant, Poisonous Plant, Physiological and Fermentation Investigations, of the Bureau of Plant Industry, U. S. Department of Agriculture. The present paper is the second of this series of studies.

The genus Penicilium was chosen for study because, owing to the investigations of Thom[1] it is now well systematized. The necessity of using pure cultures of identified molds in an investigation of this kind is obvious. Nevertheless, in most previous investigations these factors have been neglected. Many of the studies on molds deal with the action of unidentified mixtures of molds on complex substrata like maize or wheat. In many instances in which pure cultures growing upon simple media were studied, the identity of the species of mold employed can no longer be established. This is due to the fact that these investigations were, ordinarily, not conducted with the help of a trained mycologist. Such help is absolutely essential, for the difficulties of distinguishing between species are ordinarily underestimated

[1]Thom, C. H., "Cultural Studies of the Species of Penicillium," Bulletin 118, Bureau of Animal Industry, United States Department of Agriculture, Washington, D. C.

by the clinical bacteriologist. In the present series of investigations the molds were isolated by Dr. Erwin F. Smith and identified by Dr. Chas. Thom. Without such aid these studies could not have been undertaken.

In the first study of this series it was found that *Penicillium puberulum* Bainier, produces a phenolic acid of the empirical formula $C_8H_{10}O_4$, for which the name penicillic acid was suggested.[1] This acid gives a brownish red color with ferric chloride, reduces Fehling's solution and yields a deep red dye when acted upon by ammonium hydroxid. It is also somewhat toxic and antiseptic. The lethal subcutaneous dose is from .2 to .3 grams per kilo of body weight. It was not possible to identify penicillic acid with any known compound. In its general properties it resembles very greatly certain of the lichen acids found in lichens.

In the present paper a similar study upon a closely related organism, *Penicillium stoloniferum*, Thom, is reported. This organism was isolated from a specimen of spoiled Italian maize which was very kindly secured by Dr. C. H. Lavinder, of the Hygienic Laboratory of the Public Health and Marine Hospital Service while studying pellagra in Italy.

The examination of the specimen of Italian spoiled maize was undertaken because as stated in a former publication[2] it seems to differ from American spoiled maize in its behavior toward the ferric chloride reaction of Gosio. In Italy this reaction for phenolic substances is regarded as one of the most reliable tests for the determination of deterioration of maize. According to Schindler it is not highly esteemed in the Tyrol.[3] American spoiled maize when tested by the method recommended by Gosio[4] only occasionally gives the reaction. The color obtained in the few posi-

[1]Alsberg, C. L., and Black, O. F., "Biological and Toxicological Studies upon Penicillium puberulum, Bainier. Proceedings of the Society for Experimental Biology and Medicine, IX, p. 6, (1911)."

[2]Alsberg, C. L. and Black, O. F., "The Determination of the Deterioration of Maize with Incidental Reference to Pellagra. Bulletin 199, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., 1910."

[3]Alsberg, C. L. and Black, O. F. op. cit. page 27.

[4]Gosio, B.: Alterazioni Del Grantureo E Loro Profilassi. Page 35. Rome 1909. Tipografia Nazionale Di G. Bertero E C.

tive cases has always been red or brownish red, never violet or green as described by Italian investigators. Recently the test has been improved in this laboratory so as to render it more delicate. The essential improvement in the procedure as now conducted consists in extracting directly with chloroform. Fifty grams of ground corn or meal are placed in a stoppered flask and covered with chloroform. After two hours the chloroform is filtered off and concentrated to a bulk of 10-15 cubic centimeters. This is transferred to a small separatory funnel or test tube and covered with about 5 cubic centimeters of water containing a trace of ferric chloride. If substances like penicillic acid are present the characteristic color develops in the aqueous layer. Conducting the tests in this way a greater number of samples of obviously deteriorated maize show the reaction than was the case with the old test. Nevertheless a positive result seems to be less frequent in American maize than in Italian maize.

The Italian spoiled maize mentioned above gave an intense ferric chloride reaction of a violet color. Moreover, when grown on Raulin's medium it gave the same characteristic reaction. It is certainly a remarkable fact that the first sample of spoiled Italian corn examined gave the violet color described by Italian authors, whereas no American sample has been found giving a similar tint.

It was, therefore, decided to isolate, if possible, the substance responsible for the ferric chloride reaction. For this purpose the organism from Italian spoiled corn was grown on Czapek's medium and on Raulin's medium. It was found that the organism grew more rapidly upon the latter. Therefore, for the preparation of material Raulin's medium only was used.

The substance responsible for the ferric chloride reaction was isolated by the following procedure. The culture fluid and the mycelium were transferred to an evaporating dish and rendered weakly alkaline with sodium carbonate. The contents of the dish were then heated to boiling and filtered hot. The mycelium remaining on the filter was thoroughly expressed. It was then again extracted with water, rendered weakly alkaline with sodium carbonate. The combined extracts were evaporated to a small bulk over a free flame and filtered hot. To the clear filtrate a

slight excess of hydrochloric acid was added. An abundant precipitate was produced which consisted of a mixture of needle clusters and amorphous material. The precipitate was separated by filtration and washed with cold water. After drying spontaneously it was extracted with hot toluene and the hot extract filtered. Only the crystalline portion of the precipitate dissolved. The amorphous dark brown material which remained on the filter was discarded, for it did not give a color reaction with ferric chloride. The toluene extract, on cooling and spontaneous evaporation, precipitated in the form of needles, the material giving the ferric chloride reaction. These were still slightly colored but were finally obtained white, either by decolorizing with boneblack in hot toluene solution or by dissolving in alcohol and adding alcoholic potassium hydroxide to form the potassium salt which is insoluble in alcohol. This salt was then washed free from color with alcohol. From the potassium salt the free acid was recovered in the form of white needles by dissolving the salt in water and precipitating with hydrochloric acid.

The substance thus obtained consists of white needles with a melting point of 140°, uncorrected. The name mycophenolic acid is provisionally suggested for it. It is almost insoluble in water but freely soluble in alcohol, in ether or in chloroform. It is somewhat less soluble in benzene and only moderately soluble in cold, though very soluble in hot toluene. With ferric chloride it gives a violet color in aqueous solution, though its solubility in water is not sufficient to render the color intense. In alcoholic solution it gives a bright green color with ferric chloride. It does not react with Millon's reagent. It does not give Lieberman's reaction and could not be diazotized. It does not reduce Fehling's solution nor ammoniacal silver nitrate. It is fairly resistant to sodium, ammonium and potassium hydroxide and hydrochloric, sulphuric and acetic acid, not being affected by boiling in 10 per cent. solutions of any of these reagents. It does not contain water of crystallization. Its salts of potassium and sodium are very soluble in water. The former is soluble in dilute, but insoluble in absolute alcohol. The latter is soluble in absolute alcohol but may be precipitated in crystalline form by adding ether. The salt of barium is only very slightly soluble in water

and forms clusters of minute needles. The copper, lead and silver salts are amorphous and insoluble in water. In characterization of the substance the facts collected in Table I were ascertained by analysis of the free acid, by titritation of the alcoholic solution of the free acid with N/10 sodium hydroxide, using phenolphthalein as indicator, and by the determination of the barium content of the salt on ignition in platinum with sulphuric acid.

TABLE I. Analyses of Mycophenolic Acid

Weight of Substances Grams	CO_2 Grams	HO_2 Grams	C Percent	H Percent	$BaSO_4$ Grams	Ba Percent	N/10NaOH Cubic Centimeters
0.2316	0.5419	0.1315	63.81	6.30			
0.2044	0.4770	0.1161	63.64	6.31			
0.2494					0.1256	29.65	
0.1990							11.53
Average			63.725	6.305			

Calculated for $C_{17}H_{20}O_6$ Carbon 63.74 Hydrogen 6.25 %
Found Carbon 63.72 Hydrogen 6.30 %
Calculated for Ba ($C_{17}H_{18}O_6$) Barium 29.15 %
Found Barium 30.09 %

A molecular weight determination by the elevation of the boiling point in chloroform solution gave the results in Table II.

TABLE II. Ebulioscopic Determination of the Molecular Weight of Mycophenolic Acid

Weight of substance	Weight of solvent	Rise of boiling point	Molecular weight
Grams	Grams		
0.1641	30.32	0.065°	308
0.1578	30.32	0.060°	321
Average			314.5

Molecular Weight calculated for $C_{17},H_{20}O_6$ 320
Molecular weight found from titration 345.4
Molecular weight found from barium content of salt 328
Molecular weight found from boiling point elevation 314.5

The formula $C_{17}H_{20}O_6$ may therefore be assigned to mycophenolic acid. It does not readily decompose carbonates at ordinary temperatures. It is apparently a dibasic acid, or at any rate, combines with two atoms of a monovalent base. Whether the base combines entirely with carboxyl groups or with phenol groups has not been determined. The acid seems to form two series of salts. Presumptive evidence on this point was obtained by the following experiments.

0.2 grams of free acid were suspended in water and one equivalent of potassium hydroxid added. Unfortunately, this was not sufficient to put the substances completely in solution, so that a slightly greater quantity of the alkali had to be used. This solution was then treated with one equivalent of barium chloride. On standing in the dessicator a crystalline barium salt formed. This salt was evidently different from the normal barium salt which is so insoluble that it precipitates at once. It was also of different appearance under the microscope, consisting of a few small needles in clusters, which apparently were the normal salt and more abundant larger single needles, apparently the acid salt. The presence of the normal salt in small quantities under the conditions of the experiment was probably due to the fact that an excess of alkali had to be used in dissolving the substances. The barium content of this preparation was determined, 0.207 grams yielding 0.0692 grams of $BaSO_4$, equivalent to a barium content of 20.2%.

Calculated for $Ba(C_{17}H_{18}O_6$	28.1 per cent.
Calculated for $Ba(C_{17}H_{19}O_6)_2$	17.7 per cent.
Found	20.2 per cent.

Apparently, as shown by the microscope, the preparation consisted of a mixture of two salts.

It has not been found possible to identify the substances with any known compound. In very many respects it resembles the class of substances found in lichens and classed vaguely as lichen acids. To find a substance of this class in molds is not surprising since lichens are symbiotic forms composed of fungi and algae. Mycophenolic acid also resembles very greatly a substance isolated by Gosio from a species of Penicillium. The formula

$C_9H_{10}O_6$ calculated for the latter by Gosio is based on a single combustion. As far as may be judged from Gosio's records, it is probably not identical with mycophenolic acid, though resembling it greatly. However, Gosio's characterization of the substance was based on a very small quantity of material, so that it cannot be regarded as final. The chief points of difference between the substance described by Gosio and mycophenolic acid are the percentage composition and the bahavior with ferric chloride. Gosio's substance gives an intense blue color with ferric chloride in alcoholic solution. Mycophenolic acid gives a violet color in aqueous solution, while in alcoholic solution with a trace of ferric chloride it gives a violet color which becomes bright green on addition of an excess of the reagent.

In one particular mycophenolic acid resembles Gosio's substance but differs from penicillic acid. It is not toxic. Ten milligrams were dissolved in water, with the aid of a little sodium carbonate and injected subcutaneously into a mouse. No untoward effects whatever were noted. From penicillic acid, furthermore, it differs in being present chiefly in the mycelium in the early stages of growth. In old cultures it is found both in the culture fluid and in the mycelium, perhaps because with the gradual production of basic substances it is dissolved. The question whether toxic phenolic substances are found in the culture fluid or only in the mycelium is one that has been much discussed by students of pellagra. When the substances are insoluble acids with soluble salts like mucophenolic acid, their distribution is probably only a question of the reaction of the medium. When the reaction is acid they will be found in the mycelium as lichen acids incrust the lichen thallus. When the medium contains available bases they will become more or less dissolved in the medium.

With the advancing age of the culture mycophenolic acid gradually increases in quantity until under the conditions employed in these experiments the maximum yield is obtained in about two weeks. After that time the quantity present is apparently constant. When grown in rectangular quart bottles known in the trade as "long Blakes" turned on their sides in order to have a large surface and charged with about 250 cubic

centimeters of culture fluid the yield at the end of about two weeks averages per bottle about 0.07 grams of the crude acid.

Since *P. stononiferum* is found so commonly in the United States it is not easy to understand why it is so rarely, if ever, causes spoiled maize in the United States to give the ferric chloride reaction. The first explanation to present itself was that the American organism might be a different strain or perhaps a "physiological variety."

To solve this question Dr. Thom very kindly furnished a specimen of his type culture. This was grown side by side with the Italian organism. It grew rather more slowly than the latter and there were slight differences in appearance. The cultures gave a good ferric chloride reaction very similar in shade to that given by the Italian organism. When, however, the attempt was made to separate mycophenolic acid from the cultures of the American organism none could be found. In its place was found a quite different substance or mixture of substances. As this material has not yet been obtained in satisfactory crystalline form not much can at present be said of its properties.

The different biochemical behavior of the two strains from the two continents is certainly suggestive. Whether these two strains are really physiologically different can not as yet be decided. The American organism used is an old one, having been propagated by Dr. Thom in the laboratory for a number of years. Possibly this long artificial propagation has altered its behavior. It is proposed to continue the investigation of this problem by comparing the two cultures on hand with a number of new recently isolated strains.

No extended physiological studies were undertaken on *P. stononiferum*. A few observations were made incidentally. The organism always produced alcohol as shown by applying the iodoform test to the distillate. No quantitative determinations were made but the amount of alcohol formed as judged by the iodoform test seemed to be decidedly less than that produced by *P. puberulum*. *P. stoloniferum* produces a small amount of oxalic acid. To detect it the medium was concentrated to a syrup and mixed with clean sand and plaster of paris. The hardened mass was pulverized and extracted with ether in a Soxhlet apparatus.

The oxalic acid, identified by the melting point and insolubility of the calcium salt, crystallized in the extract. Oxalic acid seems to be present in somewhat larger amounts and at an earlier stage of growth than in cultures of *P. puberulum*. Finally the mycelium of *P. stoloniferum* seems to be very rich in mannitol.

Summary

From cultures of *Penicillium stoloniferum* Thom obtained from a sample of spoiled maize from Italy a new phenolic acid of the formula $C_{17}H_{20}O_6$ was isolated in crystalline form. It resembles the lichen acids, is not toxic and is one of the substances causing the ferric chloride reaction of Gosio in deteriorated maize.

THE EFFECT OF SODIUM CHLORID AND COLD STORAGE UPON THE ACTIVITIES OF PROTEOLYTIC ENZYMES

By William N. Berg

From the Dairy Division Research Laboratories, Bureau of Animal Industry, Washington, D. C.

At low temperatures and in the presence of sodium chlorid the activity of a proteolytic enzyme may be inhibited, if the amount of enzyme is small. If the amount of enzyme is large, proteolysis takes place rapidly and apparently is not interfered with by the low temperatures and sodium chlorid. These observations were made during the course of some investigations on the chemical changes taking place in cold storage butter. A detailed account of these and related investigations is soon to be published by the Dairy Division, Bureau of Animal Industry.

The Inhibiting Effect of Cold Storage and Sodium Chlorid on the Activity of Galactase in Buttermilk

Buttermilk obtained from a churning pasteurized or of unpasteurized cream may contain galactase, a proteolytic enzyme very similar in its general characters to trypsin. When buttermilk is preserved with chloroform and kept at room temperature, the galactase will slowly digest the proteins present. Some quantitative data are given in a previous publication from this laboratory.[1]

In buttermilk containing 18% of sodium chlorid placed in cold storage, (at O F or minus 18 C.) for as long as nine months, no proteolytic action was detected.

For the detection of proteolytic action, water soluble nitrogen was determined in the buttermilk before and after storage, as follows:

[1]Rogers, L. A., Berg, W. N., and Davis, B. J., Circular 189, Bureau of Animal Industry, 1912, p. 315.

Transfer 200 cc of the sample to a 500 cc volumetric flask. Dilute with water to about 450 cc. Add 20 cc 10% acetic acid. This will flocculate the casein. A cc more or less of the acid will make no difference when sodium chloride is present. Make up to the mark, filter on a 32 cm folded filter (S & S No. 595 or 588) and determine total water soluble nitrogen in two 200 cc portions of the clear filtrate.

By this method, the results for water soluble nitrogen in buttermilk containing 18% of sodium chloride and placed in cold storage for 9 months, were the same, practically, before and after storage. This indicates that the action of the enzyme was inhibited under those conditions.

The Effect of Cold Storage and Sodium Chlorid on the Activities of Proteolytic Enzymes in Sterilized Skimmilk

Digestive mixtures were prepared as follows: Skimmilk was sterilized by heating for two hours at 94, 99 C. in a steam sterilizer. The skimmilk was quickly cooled to 35 C. and to three 3 liter portions in separate containers, 540 grams of sodium chlorid was added, making the salt concentration approximately 18%. To one of these mixtures there was added 3 grams of pancretin, dry, U. S. P., to the second, 3 grams of pepsin, dry, U. S. P. and to the third 15 grams of a dry proteolytic enzyme preparation obtained from cultures of lactic acid bacteria which also digested protein. The enzyme was precipitated from the cultures (by L. A. Rogers) with alcohol in the manner usually used for such preparations. The enzyme preparation was tested before hand and found to liquefy gelatine.

Controls were likewise prepared, which differed from the before described mixtures only in the fact that the enzyme preparations were added to them while the shimmilk was near the boiling point.

The mixtures were kept in sealed cans having a capacity of 1 liter. Each can contained 600 cc of the sample, and was kept in cold storage for 9 months at 20 F (minus 7 C). Water soluble nitrogen was determined in these mixtures before and after storage by the method used for galactase.

It was found that under these conditions, the pancreat in (trypsin) was very active, pepsin acted a little more slowly while the bacterial enzyme preparation showed very little, if any activity. The controls showed no change. In the trypsin mixture 2-3 of the total nitrogen present was rendered water soluble. In the pepsin mixture 1-3 of the total nitrogen was rendered water soluble. When these mixtures were allowed to stand at room temperature, further digestion took place. In the trypsin mixture practically all the protein became water soluble.

It is to be noticed that both pepsin and trypsin acted vigorously in different portions of the same substrate.

However, the claim is not made that sodium chlorid does not exert an inhibiting influence. Under certain conditions it does. Experiments were made in this laboratory in the spring of 1909, in which the speed of digestion of casein in several pepsin-acid solutions was compared with that in the same solutions to which 20 grams of sodium chlorid had been added to 100 cc of acid solution. The presence of the salt almost completely inhibited the action of the pepsin-acid during the experiment—40 minutes' digestion. It is of course probable that digestion would have taken place had the digestion period been several months. The method of comparing speeds of digestion was that described by Gies.[1]

The results show that whether sodium chlorid does or does not inhibit proteolysis depends upon the amount of enzyme, to a very large extent.

[1]Berg, W. N., and Gies, William J., Journal of Biological Chem. Vol. 2, pp. 489-546, 1907.

FATTY ACID ESTERS OF GLUCOSE

By W. R. Bloor

(From the Laboratories of Biological Chemistry of Washington University, St. Louis, Mo.)

This paper is a preliminary report on the preparation and properties of a new class of compounds—the fatty acid esters of glucose. The interest of compounds of this type is threefold: (1) the relationship which has been shown to exist between carbohydrates and fats in metabolism; (2) the possibility of the natural occurrence of these and similar compounds; (3) the possible usefulness of such compounds in the study of fat metabolism. A relationship between fat and carbohydrates in metabolism has been repeatedly noted. In the absence of a sufficient supply of available carbohydrate, as in starvation or severe diabetes, the fats are incompletely burned and the unburned residues are excreted as B-oxybutyric acid and its derivatives, diacetic acid and acetone. The fact has been crystallized in the statement attributed to various investigators that " fats can burn only in the fire of the carbohydrates." The acidosis may be decreased or made to disappear if carbohydrates can be fed and utilized.

That the condition may be reciprocal, i.e., that the fats, under certain conditions may help in the metabolism of the carbohydrates has, so far as I know, never been suggested but it seems something more than a coincidence that the carbohydrate of oats—the grain which has the highest per cent. of fat of all ordinary grains—should be the best utilized by diabetics.

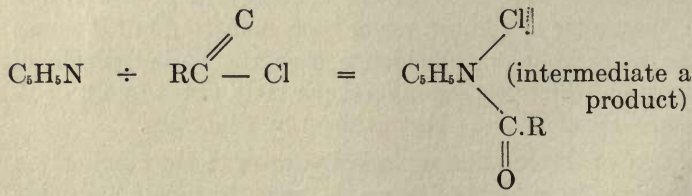
The nature of the relation of carbohydrates to the combustion of fats has been the subject of many theories, the most reasonable of which is that the glucose acts as a catalyser, either by furnishing readily available oxygen, or by the formation of a compound with the fatty acid which is more readily burned than the fatty acid alone.

Glucose esters of the fatty acids have so far not been found in nature. As may be seen from a study of the properties of the compounds already prepared they are so much like the fats and lipoids in their solubilities, etc., that they may well have escaped detection. Compounds of galactose with fatty acids and other substances are well known to occur in the brain substances (cerebrosides).

Compounds of this sort are of interest also because they may be useful in solving the problem of the absorption and transportation of fats. If absorbed unchanged they could be readily recognized in the chyle and if injected into the blood stream could be readily traced. Some work along this line has already been done using analogous compounds—the mannite esters of the fatty acids.[1]

Glucose esters of butyric acid (di-butyrate) and stearic acid (di-stearate) have been prepared by Berthelot[2] by direct combination at high temperatures. The yield was slight and his description of the compounds is limited to their physical appearance and two or three solubilities. Because of the small yield and the instability of glucose at high temperatures this method does not lend itself to the preparation of the compounds in large quantity.

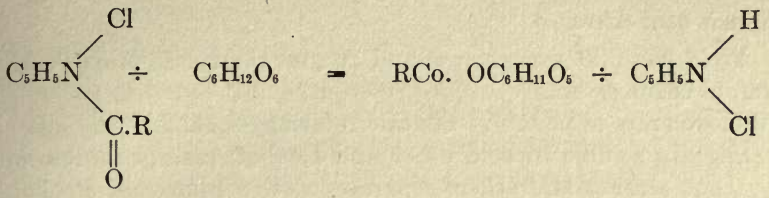
The synthesis adopted depends on the action of the chlorides of the fatty acids on glucose in solution in pyridin, the pyridin acting both as solvent and catalyser somewhat as follows[3]:



[1]Bloor, Journal of Biological Chemistry, XI, p. 429.

[2]Berthelot Annals de Chemie et de Physique, (3) 60. 96. (quoted from Beilstein Handbuch der organischen Chemie, 3rd ed. vol. 1, p. 1049).

[3]Einhorn and Hollandt-Liebig's Annalen, 301. 95 (1898).



Process.

25 grm. of dry glucose is dissolved with the aid of heat in five to ten times its weight of dry pyridin, the solution cooled, and an equimolecular amount of the chloride of the fatty acid added in small portions with cooling. The mixture is allowed to stand over night, then poured into iced dilute sulphuric acid. The esters separate and float on top, and are freed from the liquid (in the case of the higher fatty acids) by filtering on a suction funnel. The mass is then boiled out several times with water, until it is free enough from electrolytes to form a colloidal solution. It is caused to separate from the colloidal condition by the addition of sodium sulphate, let cool and the solid cake removed and dried. The mixture is first fractioned with ether to separate the higher esters and then with alcohol.

The following is a brief description of the compounds which have been separated. Because of the great difficulty in making the separations the data given are regarded as only approximately correct.

General properties of the esters.

The compounds all reduce Fehling's solution and are optically active, the optical activity being less than that of glucose. They form colloidal solutions with water (best made by pouring the hot alcoholic solutions into water). They are readily saponified by acids or by alkalies (watery or alcoholic). They do not ferment with yeast. The presence of glucose was shown by saponification with alcoholic hydrochloric acid and preparation and identification of the osazone. Some of the compounds possess the property, in common with glucose, of forming sodium compounds when treated in alcoholic solution with sodium ethylate.[1] The compounds precipitate out as a gummy mass.

[1]Honig, Rosenfeld: Berichte der Deutsch. Chem. Gesellsch. 10. 1871.

Stearic acid esters.

Yield from 20 gm. glucose and 30 gm. stearyl chloride — 35 gm. of crude esters.

Monostearate m.p. 110°. Specific rotation ÷ 36.25°.

Slightly soluble in cold ether and alcohol; readily soluble in hot alcohol and ether; separates slowly from cold alcohol. The separation is hastened by the addition of ether.

Distearate m.p. 90-95°. Dextro rotatory

Slightly soluble in cold ether and alcohol; readily soluble in them hot; separates quickly from alcohol on cooling.

Tristearate m.p. 60°. Specific rotation ÷ 12.00°.

Soluble in cold ether; soluble in hot alcohol from which it separates on cooling.

Lauric acid esters.

Yield from 25 gm. glucose and 26 gm. lauryl chloride = 38 gm. crude esters.

Monolaurate—well crystallized in shining rhombic plates. m.p. 110°. Specific rotation ÷ 30°.

Slightly soluble in cold alcohol and ether; readily soluble in them hot. The separation from alcohol in the cold is aided by the addition of ether.

Dilaurate α m.p. 55°. Specific rotation ÷ 21.8°.

Somewhat soluble in cold ether. Soluble in hot alcohol from which it separates on cooling.

Dilaurate β m.p. 33°. Specific rotation ÷ 30°.

Readily soluble in cold ether. Soluble in hot alcohol from which it separates on cooling.

Butyric acid esters.

Yield from 25 gm. glucose and 12 gm. normal butyryl chloride ÷ 3 gm. of mixed esters.

This synthesis is evidently not adapted to the preparation of the butyric esters. The ester mixture agrees with Berthelot's[1] description of the dibutyrate. "Very bitter liquid, somewhat soluble in water, very easily in alcohol and ether." Strongly dextro rotatory (÷ 31°).

[1]Berthelot: loc. cit.

Animal experiments.

The material for the animal experiments was prepared from the fatty acids of cocoa-nut oil after separation of oleic, palmitic and stearic acids.[1] The fatty acid mixture used had a mean molecular weight of from 200-210 and a melting point of 30°-36° C. depending on the sample. Acid chlorides were prepared from this product by the method of Krafft and Burger.[2] The glucose esters were prepared from the acid chlorides in the way described above. The ester mixture so prepared and which was used for the animal experiments had a m.p. of 41° but remained soft and sticky at 30°. It was readily and completely soluble in cold ether.

Specific rotation ÷ 21°.

Feeding experiments.

The animal used for the feeding experiments was a cat, weight 2 k.

5 gm. of the ester mixture together with 25-30 gm. of lean meat, 5 gm. cotton seed oil and 2-3 gm. bone ash was fed every third day. In the intermediate days and on the first two days of the experiment the animal received the diet without the ester and containing 1-2 gm. of wood charcoal in place of the bone ash. It was hoped in this way to get sharply divided feces corresponding to the feeding periods. As may be seen from the results it was not possible in this experiment. Charcoal feces very often had a core of bone ash feces and the two were otherwise so mingled that only an approximate separation was possible. The feces from each period were extracted with ether in a Soxhlet extractor for 3-4 hours and the extract examined polarimetrically for unabsorbed esters.

Preliminary period. Two days.

50 c.c. ether extract, reading in 1 dcm. tube = ÷ 0.03° corresponding to a weight of ester of 0.10 gm.

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[1]For a description of the method of separation see Bloor: Jour. Biol. Chem. XI, p. 421 (1912).

[2]Krafft and Burger: Berichte der Dutsch. Chem. Gesellsch. 17, 1378 (1884).

This figure was used as correction in the other periods.
First two ester periods (bone ash) extracted together.
65 c.c. of ether extract
Polariscope reading in 1 dcm. tube + 1.05°
Corresponding to a weight of ester of 3.40 gm.
Correction for blank 0.10 gm.
Corrected weight 3.30 gm.
First two control periods (charcoal)
28 c.c. of ether extract
Polariscope reading in 1 dcm. tube + 0.20°
Corresponding to a weight of ester of 0.28 gm.
Correction for blank (4 days) 0.20 gm.

Corrected weight 0.08

Summary of first two periods	Ester fed	10 gm.
	Ester recovered	3.40 gm.
	Absorbed	6.60 gm.

Per cent. absorption 66%.

Third period (some diarrhoea)
Ester feces 98 c.c. of ether extract
Polariscope reading 0.50°
Corresponding to a weight of ester of 2.45 gm.
Correction for blank 0.10 gm.

Corrected weight 2.35
Control feces 65 c.c. ether extract
Polariscope reading 0.03°

For third period	Ester fed	5 gm.
	Ester recovered	2.35 gm.
	Absorbed	2.65 gm.

Per cent. absorption 53.0

Fourth period
Ester feces 120 c.c. of ether extract
Polariscope reading in 1 dcm. tube 0.12°
Corresponding to a weight of ester of 0.72 gm.
Correction 0.12 gm.

Corrected weight 0.60 gm.

Control feces 40 c.c. ether extract
Reading in 1 dcm. tube 0.20°
Corresponding to a weight of ester of 0.40 gm.
Correction for blank 0.10 gm.

Corrected weight 0.30 gm.

For fourth period	Ester fed	5 gm.
	Ester recovered	0.90 gm.
	Absorbed	4.10 gm.

Per cent absorption 82%

Summary of experiments:

First two periods	Absorbed	66%
Third period	"	53% (diarrhoea)
Fourth period	"	82%
Average absorption		67%

Injection experiments.

The material used for the injections was a colloidal solution of the esters in water made by pouring a hot alcoholic solution into water, filtering hot and boiling until the alcohol had evaporated and the solution had reached a concentration of about 10%. The milky suspension so obtained could be flocked out by the addition of acids or of strong salt solutions, but could be diluted with several volumes of normal salt solutions without separation taking place for several hours. The ester mixture used in preparing the solution was prepared for use by washing the ether solution with dilute alkali until free from fatty acids, then several times with distilled water.

Intraperitoneal injections.

The animal used was a young rabbit (Belgian hare) weighing 1.5 K. Two injections were made on succeeding days of (1) 5 c.c. of suspension containing 0.5 gm. of ester and (2) 10 c.c. of solution containing 0.9 gm. of ester. The animal showed no bad effects. Postmortem[1] examination two weeks later showed that part of one injection had lodged between the muscular coat and the peritoneum. Microscopic examination of the cheesy mass

[1] I am indebted to Doctor W. S. Thomas of the Department of Pathology of this School for the postmortem examination.

showed it to consist practically entirely of leucocytes. Extraction of the substance with ether showed that the ester had disappeared. Below the mass, the peritoneum was united to the intestines by many adhesions. Scattered through the intestines and also in the diaphragm and one edge of the liver were many encapsulated masses of the same nature. Except for the above all organs were normal.

Intravenous injections.

Made on rabbits.

Experiment I. Large (3 k.) rabbit with light brown spots. Ten c.c. of solution containing 1 gm. of ester was injected into the lateral ear vein during about 30 minutes. After the injection the animal appeared normal and was put back into the cage. Ten minutes later (about 40 minutes after beginning injection) it was down and kicking convulsively and one minute later respiration had ceased, although the heart continued to beat for a short time longer. Autopsy showed a marked injection of the vessels on the left side of the pons. Otherwise no abnormality.

Experiment II. Young rabbit weight 1.5 k. (the same one as was used for the intraperitoneal injections above). First injection—10 c.c. of solution containing 0.8 gm. of ester injected into the lateral ear vein, the injection lasting fifteen minutes. After the injection the animal behaved normally and showed no immediate bad effects; nevertheless although fed liberally it rapidly lost weight during the next few days. Second injection, five days later—6 c.c. of solution containing 0.9 gm. of ester injected into the median ear vein, the injection lasting 15 minutes. After the injection the animal appeared dull and listless. No other signs were noted for the next three hours but it died during the night.

Discussion of the animal experiments.

The feeding experiments show that the glucose esters are quite well utilized in the intestine. The injection experiments, although too few in number to allow accurate deductions, indicate that the substance is probably not well borne when injected either intraperitoneally or intravenously. In the peritoneum it seems to act as an irritating foreign body while intravenously even if we regard the death of the first animal as an accident its effects on the animal are injurious.

QUANTITATIVE OXIDASE MEASUREMENTS

By Herbert H. Bunzel[1]

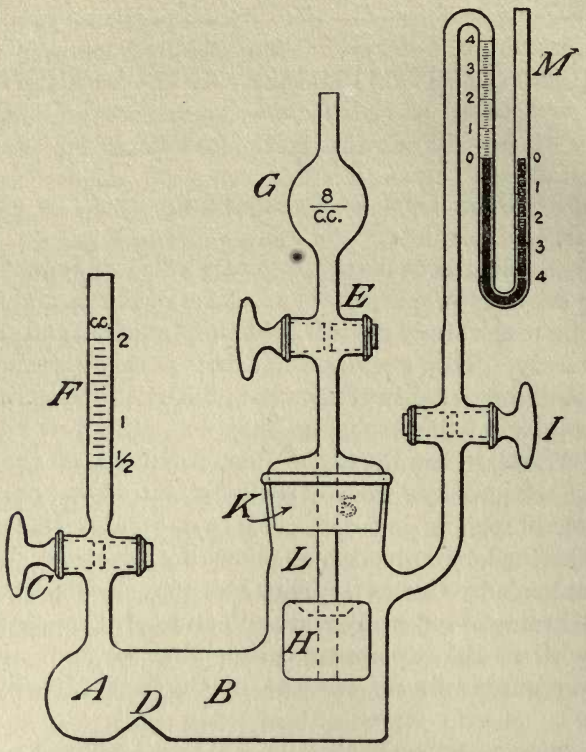
U. S. Dept. of Agric., Wash., D. C.

The very voluminous literature on the rôle and importance of oxidizing enzymes in many vital processes of plants and animals makes a thorough study of their behavior, function, and distribution necessary. They play an important part in certain pathological conditions, and in numerous industrial, and agricultural problems. As specific examples may be mentioned the work done by Woods, in the Bureau of Plant Industry, on the mosaic disease of tobacco, the work of Palladin and his school on the respiration of plants, and the casual relationship between the oxidases and color production as shown for plants by Palladin, and for animals by Gortner. They also play an important part in the darkening of tea, and the manufacture of Japanese lacquer.

Nearly all of the experiments made thus far have not been carried on quantitatively because of the lack of satisfactory methods.

The method described in this paper is based on oxygen absorption. For this reason a constant temperature is essential. The apparatus in which the oxidations are carried out is shown in the text figure. Eight cubic centimeters of the solution of the substance to be oxidized are measured in the pipette G and allowed to run into the compartment B. The plant juice, the oxidizing power of which it is desired to study, is measured in pipette F and run into compartment A. Basket H holds 1 cc. of normal sodium hydroxide to absorb the carbon dioxide formed in the process; M is a manometer charged with mercury to indicate the pressure within the oxidase apparatus. The whole apparatus is clamped to a specially constructed shaking machine. In the

[1]From the Bureau of Plant Industry, U. S. Department of Agriculture, Office of Drug Plant, Poisonous Plant, Physiological and Fermentation Investigations.



APPARATUS FOR MEASURING OXIDASE ACTION

air-thermostat the temperature is brought to 37° C. and maintained at that point to within 0. 1° throughout the experiment. Half an hour after the temperature of 37° is reached, all stopcocks but one are closed, and the shaking machine set in operation. The plant juice mixes with the oxidizable material and the reaction begins. From time to time the shaking is interrupted and the manometer is read. In the course of several hours the oxygen absorption is completed, as indicated by no further change of pressure within the flask. The ultimate reading expresses the oxidase content of the juice or extract with respect to the particular substance used. As a unit an oxidase solution is chosen of such a strength that one liter of it will be capable of bringing about the consumption by pyrogallol of the equivalent of one gram of hydrogen.

Hitherto pyrogallol, tyrosin. hydrochinone, guaiacol, benzidine, and alphanaphthol have been investigated. The concentration of the material to be oxidized has no effect on the end result provided it is used in excess. The carbon dioxide produced is absorbed by the alkali in the basket and may be determined at the end of the experiment by means of a special apparatus devised for the purpose. The result obtained is directly proportional, or at least nearly proportional, to the concentration of the oxidase present.

An application of the method to potato juice is given in Table 1. In each one of the experiments 8 cc. of a 1% Pyrogallol solution and 2 cc. of potato juice were used.

TABLE I.

Time of Manometric Reading	Manometric Reading	
P. M.	a.	b.
1:30	0.00	0.00
1:40	1.00	0.90
1:50	1.40	1.20
2:00	1.55	1.42
2:10	1.62	1.55
2:20	1.76	1.70
2:30	1.80	1.80
2:40	1.80	1.82
2:50	1.82	1.83
3:00	1.82	1.85

For the sake of studying the applicability of this method to the determination of oxidases in juices of plants other than potatoes, some experiments were carried out on sugar beet leaves. The Division of Cotton and Truck Diseases of the Bureau of Plant Industry, Department of Agriculture, has for some years been investigating the curly-top disease of sugar beets. The writer was able to obtain for experimental purposes fresh samples of sugar-beet leaves affected by this disease to a striking degree, and also samples of normal beet leaves. All of the beets, of which the leaves were examined were grown in

a green-house and therefore were subjected to practically uniform conditions. The juice was obtained by grinding the leaves up in a meat chopper and pressing the juice out of the pulp through a silk cloth. The results obtained are summarized in Table II.

TABLE II.

Juice used	Manometer readings expressed in centimeters of mercury
1. Juice of normal beet leaves	1.16
2. Juice of normal beet leaves	1.07
3. Juice of diseased beet leaves	5.61
4. Juice of diseased beet leaves	4.30
5. Juice of normal beet leaves	1.10
6. Juice of normal beet leaves	1.17
7. Juice of diseased beet leaves	2.72
8. Juice of normal beet leaves	1.19
9. Juice of normal beet leaves	1.21
10. Juice of diseased beet leaves (showing slight symptoms only)	1.51
Mean absorption in experiments with juice of normal plants	1.15
Same expressed in terms of units	1.66
Mean absorption in experiments with juice of diseased plants	3.54
Same expressed in terms of units	5.09

Experiments given in Table II show a very striking difference between the juice of the normal and that of the diseased beet leaves. In all of the experiments the oxidase content as indicated by the oxygen absorption of the pyrogallol in the presence of the juice is markedly greater in the diseased than in the healthy leaves. The oxidase content of the normal leaves seems to be fairly constant, while the juice of the curly-top beet leaves shows wide variations. The leaves used in experiment three gives about five times as high a figure as normal leaves, while

the leaves chosen in experiment ten show a variation of only 25 per cent. from the normal. It is very interesting to note that the deviation in oxidase content of the pathological leaves, as measured by the method described, runs parallel with the appearance of the leaves. The plants used in experiment three showed very marked signs of curly-top, the leaves being small and shriveled, and the hairy roots abundant, while the diseased beet used in experiment 10, which showed a relatively low oxidase content, but still higher than normal, had only a slight curling of the leaves.

Although these results are quite conclusive in showing existing differences in the oxidase mechanism of the healthy and diseased sugar-beets grown in the green-house, it is not justifiable without further experimentation to apply these results to conditions in the field. Where sugar-beets are grown on a commercial scale an entirely different and widely varying environment prevails. At the suggestions of Mr. W. A. Orton of the Division of Cotton and Truck Diseases a trip was undertaken to Ogden, Utah, where tons of sugar-beets are harvested every year. The writer spent the month of August, 1911, at the beet-sugar factory of the Amalgamated Sugar Company, where all of the experiments described in this paper were carried out.

The juice of the leaves and roots was obtained by chopping and pressing out through a silk cloth as before. Table III gives a summary of the results.

TABLE III.

	Material from which juice was obtained	Activity of juice expressed in terms of units
1.	Healthy, large outer leaves	0.191
2.	Curly-top diseased, small inner leaves of same plants	0.381
3.	Root of same plant	0.252
4.	Leaves of small plant, retardation of growth, unknown, no symptoms of curly-top	0.367
5.	Upper half of root of same plant	0.086
6.	Lower half of same root	0.158

	Material from which juice was obtained	Activity of juice expressed in terms of units
7.	Leaves of large and healthy plant adjoining small one	0.201
8.	Upper half of root of same plant	0.172
9.	Lower half of same root	0.288
10.	Leaves of small plants, growth retarded by drought. No symptoms of curly-top	0.230
11.	Uppermost third of root of same plant	0.130
12.	Lowest third of same root	0.403
13.	Leaves of large and healthy plants, collected on the same field and the same time as 10	0.144
14.	Uppermost third of root of same plant	0.173
15.	Lowest third of same root	0.360
16.	Leaves of curly-top plants showing marked symptoms	0.288
17.	Uppermost thirds of roots of same plants	0.259
18.	Lowest thirds of same roots	0.475
19.	Lowest fifth of roots of curly-top plants	0.504
20.	Uppermost fifth of same roots	0.317
21.	Leaves of curly-top plants grown from beets which had been siloed and showed no symptoms of the disease in the year previous. "Trotze"	0.496
22.	Roots of same plant	0.216
23.	Leaves of plants similar to those described in 21	0.446
24.	Roots of same plants	0.183
25.	Leaves of curly-top plants with seed stem, carrying seeds	0.403
26.	Roots of same plants	0.324
27.	Leaves of "Trotze" plants but apparently healthy otherwise	0.446
28.	Roots of same plants	0.237

	Material from which juice was obtained	Activity of juice expressed in terms of units
29.	Leaves of a large healthy plant	0.256
30.	Leaves of a large healthy plant	0.266
31.	Leaves of healthy looking seed-carrying plant	0.288
32.	Uppermost third of root of same plant	0.331
33.	Lowest third of same root	0.381
34.	Leaves of plant of similar type as 31	0.230
35.	Upper half of root	0.144
36.	Lower half of roots	0.237
37.	Leaves of healthy, very young plants, leaves only 3-8 cm. long.	0.180
	Mean oxidase activity of juice obtained from leaves of healthy and developed plants (1, 7, 13, 29, 30, 31, 34)	0.225
	Mean oxidase activity of juice obtained from leaves of plants, whose growth has been retarded (2, 4, 10, 16, 21, 23, 25, 27)	0.382
	Mean oxidase activity of juice obtained from roots of healthy and developed plants (8, 9, 14, 15, 32, 33, 35, 36)	0.261
	Mean oxidase activity, of juice obtained from roots of plants, whose growth has been retarded (3, 5, 6, 11, 12, 17, 18,19, 20, 22, 24, 26, 28)	0.265

The experiments summarized in Table III fully corroborate the results obtained with curly-top diseased sugar-beets grown in the green-house. The leaves of the curly-top plants have an oxidase content about twice as great as the healthy and normally developed ones. No differences could be detected between the roots of the two kinds of plants. An abnormally high oxidase content of the leaves was also shown in other plants, the growth of which has been retarded. Whether the plants are stunted by excessive watering, drought, or for other unknown reasons, the oxidase content in the leaves is much higher than in the normal and healthy plants. The increase in oxidase concen-

tration in the leaves is not a mere function of their size, inasmuch as very young normal leaves do not exhibit this characteristic. If the condition of the plant is such that only one of its functions, the development of seed in bi-ennially-grown beets is inhibited, the oxidase content is also high. Such plants are called "Trotze." The most general conclusion to be drawn from these observations is that in sugar-beet plants where an abnormal retardation of growth has occurred, this is accompanied by an increase in the concentration of oxidases in the leaves or a change in the juice of the latter by which the pyrogallol oxidizing oxidase becomes more active.

Such an increase in the power of the juice to bring about or hasten oxidation under pathological conditions has been observed before. Woods found it in connection with a disease of tobacco, Sorauer in connection with the curly-top of potatoes. Future investigations will show whether or not the oxidases which have been studied by former investigations and by the writer are the same or directly related to those which Palladin and his school find so important with respiration in plants. If they are, it is probable that an increase in their concentration leads to increased combustion in the cells. One would then be tempted to look at such plants in a state of "fever."

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LES PHOSPHATES ET LE SON DE FROMENT DANS L'ALIMENTATION ANIMALE

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Depuis déjà une trentaine d'années, le public s'est engoué pour l'acide phosphorique et les phosphates. Il les considère dans l'alimentation animale comme des excitants éminents de l'assimilation.

Pendant longtemps cependant on a estimé que leur rôle n'était à prendre en considération que comme appoint, et lorsqu'ils faisaient plus ou moins défaut dans l'aliment naturel. Enfin, une école a nié quelque temps que leur rôle était nul autrement qu'à l'état de combinaison organique.

En 1904, M. Cozelle, Médecin-Vétérinaire, praticien doublé d'un vrai savant dont les travaux les ont été couronnés par nos deux grandes académies et ensuite par la Société des Agriculteurs de France, a apporté de précieux arguments pour éclairer le sujet. Ses expériences bien conduites sur des veaux et des vaches laitiéres établissent nettement que l'acide phosphorique et mieux encore les phosphates, hâtent la croissance des jeunes ruminants; mais que le résultat est encore plus sensible sur les sujets en bas âge. Enfin, ces mêmes expériences démontrent que les phosphates donnés sous la forme d'os de veaux séchés et pulpés à ces jeunes ruminants sont ceux qui ont amené les résultats les plus nettement avantageux.

Cette supériorité des os sur leur poids correspondant de phosphates minéraux est des plus rationnelles. Avec es phosphates chimiques, en effet, on n'apporte à l'organisme qu'un seul et unique de ses constituants principaux; tandis qu'avec les os eux-mêmes, c'est toute la série de ces constituants connus et inconnus aussi nombreuse et variée soit-elle. On comprend

aisément qu'en fournissant à la force vitale et en proportion exacte, tout ce qu'il faut pour reconstituer l'os, elle s'en acquitte à merveille.

Cependant l'usage de poudre d'os verts, à la campagne surtout, nous parait bien aléatoire; vu qu'en se désséchant les os se putréfient vite et toujours peu ou prou et qu'il n'est pas indiffféent de donner fréquemment à un herbivore surtout, un levain de putridité animale.

Par ailleurs, l'auteur apporte des preuves irréfutables de l'action heureuse des phosphates sur le volume de lait produit par les vaches laitières. Il est très sobre, il est vrai, sur les modifications qu'en éprouve la qualité, mais on conçoit mal qu'elles ne soient pas également favorables.

A côté de l'aliment phosphaté complexe qu'est l'os, on nous permettra de placer le son de froment et ses variétés. Celui-ci a l'avantage d'être de préparation facile, de conservation aisée, d'emploi presque banal.

Pour le vulgaire, le son-'est qu'un organe de protection du grain, son enveloppe adhérente, sa peau. Quand, par les procédés modernes, les meuniers l'enlèvent par grandes plaques pauvres en partie farineuses, c'est le gros son. Le nom de petits sons est réservé aux parties plus menues, plus déchirées, moins bien détachés et ou adhère un peu plus des tissues sous jacents. Enfin, les repasses sont constituées par des cassures du grain ou un éclat de son est opiniatrement resté adhérent à un fragment superficiel de l'amande farineuse. C'est une sorte de blé concassé, mais sans farine ni parties tendres centrales.

Au point de vue de l'alimentation animale, la composition de ces issues et leur rôle phosphatogène n'est pas sans intérêt.

En dehors de éléments alibiles, on y trouve d'abord une quantité notable d'oxydase, diatase soluble particulière qui a pour rôle de véhiculer l'oxygène de l'air sur les éléments avec lesquels elle est en contact immédiat par l'intermédiaire de l'eau. C'est pourquoi quand on mouille le son, toutes les parties humidifiées brunissent. C'est à cause de sa présence dans les parties du son, même les plus ténues, que les farines de queues donnent du pain bis.

Dans toutes les parties du grain où se trouve cette oxydase et dans des proportions absolument parallèles se trouve du

manganèse. Ce fait est à noter en passant, à cause de la coexistence annoncée entre ces deux substances aux allures originaires communes.

C'est probablement dans cette même région du grain que l'on trouvera tôt ou tard ces éléments rares qui paraissent nécessaires à l'évolution normale de tous les êtres vivants animaux et végétaux tels que l'iode, l'arsenic, le bore, le zinc et autres. Dans tous les cas, il est aisé de constater que c'est ici que se concentrent le fer, la chaux, la magnésie, la potasse et par dessus tout le fluor et l'acide phosphorique, etc.

Mais cet ensemble a ceci de particulier, c'est que la majeure partie de ces éléments minéraux affectent une forme organique; si bien, par exemple, que les soi-disant phosphates des cendres du son, refusent aux 3-4 au moins de se laisser précipiter par la magnésie anmomiacale, lorsque sans calcination on les a séparés du son lui-même par l'eau chlorhydrique.

Ces diverses considérations nous expliquent que physiologiquement le son est la dernière des réserves alimentaires dont la nature a doté le grain de froment pour nourrir l'embryon, puis la plantule, lorsque dans son évolution cette derniére prend déjà une part de sa nourriture dans l'air et dans le sol. Et si cette même nature lui a donné cette texture cornée et cette résistance relative aux agents de dissolution ordinaire, c'est, d'abord, à cause de son rôle physique de prorection du contenu; c'est, ensuite, pour conserver au jeune végétal un dernier morceau de pain jusqu'au jour de son émancipation maternelle.

Voilà pourquoi, lorsqu' on incinère parallèlement les diverses parties du grain de blé, telles que la meunerie moderne sait si bien les séparer, on trouve, que selon le nombre des passages du grain aux cylindres la dose des cendres forme une proportion arithmétique progressive de phosphates. Ainsi elle commence à 0. 480% pour la farine de première et elle arrive à 5.90% pour les gros sons en passant par 0.960 pour la farine entière et à 3.50% pour lesrepasses.

Or, dans ces cendres, si on dose à l'état d'acide phosphorique total les éléments phosphorés transformés, on trouve qu'ils vont de 0.175% dans la farine de première et à 3.15% dans le son en passant par 2.10 dans la repasse et 0.23 dans la farine entière.

Ces nombres traduits en *phosphate tricalcique des os* deviennent:
Pour la farine de première 0.38%—Pour le son 6.87%.
Pour la repasse 4.58% et la farine entière 0.50.

Ces considérations et ces faits traduits par des nombres nous disent pourquoi, depuis des siècles assurément, les éleveurs font inconsciemment bon usage des sons et répasses de froment pour compléter la pauvreté alimentaire de certaines pitances végétales. Ils nous indiquent comment, parmi les herbivores, les ruminants en profitent plus que d'autres; pourquoi pour les porcelest on se trouve bien de faire cuire lessons; pourquoi les repasses favorisent la quantité au moins du lait des vaches; et pourquoi, enfin, ces mêmes sons ou repasses mélangées aux patées des jeunes granivores accélèrent la croissance de leur âge et diminuent leur mortalité quand ils sont encore dans le seul duvet.

ENTRETIEN DU TISSU DENTAIRE PAR UNE ALIMENTATION APPROPRIEE

Par M. le Dr. P. Carles de Bordeaux

Pour que les dents se forment chez l'homme comme chez les autres mammifères, à l'époque de la première et de la deuxième dentition, il est indispensable évidemment que tous les éléments minéraux et peut être même une part des organiques dont elles sont formées, préexistent dans les aliments. Il faut en plus que ces éléments aient été rendus assimilables dans le tube digestif, afin que les humeurs puissent les véhiculer jusqu'à la matrice de la dent.

Comme les dents sont constituées aux 2-3 environ par des substances minérales, dont le phosphate de chaux constitue la partie prépondérante, c'est généralement ce dernier que les dentistes prescrivent dans les défauts de nutrition du système dentaire. Nous craignons qu'en le conseillant ils ne songent pas assez aux différences d'assimilabilité que ce phosphate possède selon son genre de préparation, son origine, sa constitution moléculaire organique ou minérale; pas plus qu'aux impuretésqui accompagnent bien souvent celui du commerce.[1] Ce détail mériterait cependant d'être pris en considération, car il peut constituer une cause d'inassimilabilité générale, ainsi qu'on le verra plus loin. (Expériences de Raulin).

Mais en admettant que sur tous ces points ce phosphate soit irréprochable, il n'en est pas moins vrai qu'il serait incapable à lui seul de faire des dents, puisqu'il se trouve en outre et invariablement dans ces organes de nombreuses espèces minérales différentes connues et même probablement inconnues.

Dans cet ensemble, le phosphate de chaux n'est donc qu'une dominante; et, par cela seul que ses proportions et même son assimilabilité dans l'alimentation seraient assurées, il ne s'ensuit

[1] Il contiendrait parfois jusqu'à 0.66% de plomb, ce qui rendrait dangereuse l'administration prolongée de ce sel (A. Gubler-commentaires du Codex p. 685).

pas que le recrutement des autres éléments le soit assui. Sans doute, à cause de leur quantité plus faible et même quelquefois infime, on a pris l'habitude de considérer ceux-ci comme accessoires; mais c'est là une faute, ainsi que les points suivants vont l'établir.

Raulin, de l'Ecole Pasteur a démontré le premier, avec toute la vigueur scientifique désirable, que dans un aliment complet les éléments chimiques constituants ont tous une valeur égale, indépendants de leur proportionnalité. Ceci veut dire que non seulment les espèces chimiques en minorité numérique ont autant d'influence que les autres sur la croissance normale du sujet, mais qu'elles en ont même quelquefois d'avantage. Tel est le cas du zinc, dant la présence de traces dans un aliment élémentaire complet *décuple* une récolte ordinaire d'aspergillus. Tel est, dans le sens opposé, le rôle de la présence de traces d'argent bien moindrex encore qui s'opposent *absolument* à l'assimilabilité *de l'ensemble des autres éléments constituants*[1]

Dans le même ordre d'idées, voici un autre fait cité par Pasteur lui-même[2].

Quand on veut nourrir de jeunes levures, le meilleur aliment minéral à leur fournir est représenté par les cendres de levures vieilles; mais à la condition absolue que ces cendres, aient été simplement brûlées et non pas frittées. Si, en effet, elles ont simplement chauffées au rouge jusqu'à fusion, elles deviennent aussitôt un mauvais aliment. Cette différence tient uniquement à ce qu'elles perdent une faible partie de leursalcails, sous l'influence de l'intensité du feu, l'aliment, complet avant la fusion, est, au contraire, devenu incomplet à la suite.

Cette direction d'idées a plus récemment encore été corroborée par Sachs, A. Gautier, Baumann, Robin, Bertrand. Ces savants ont démontré que les animaux supérieurs comme les inférieurs et comme aussi les végétaux ont besoin de traces de substances à actions physiologiques intenses—arsenic, iode, bore, manganse, aluminum—mais mitigées par la forme organique que la nature sait leur donner et dont la science essaie de les revêtir depuis peu.

[1]Duclaux—Chimie biologique 1883, p. 206 ou encore—Traité de microbiologie 1898 T. 1 p. 181.

[2]Duclaux—Chimie Physiologique 1883 p. 327.

Bertrand a écrit naguère[1]: Tous les éléments constitutifs de la matière vivante sont nécessaires; tous concourent à la formation des liquides et des tissus dont l'individu se compose. L'insuffisance d'un seul de ces éléments peut entrainer la diminution de tous les autres et provoquer par suite un arrêt général de la croissance. Le principe de la synergie des éléments prend donc une grande importance au point de vue du choix de certaines médications, etc.

Tous ces faits peuvent se résumer ainsi:

Dans l'alimentation, c'est l'assimilation des petits qui entraine l'assimilation corrélative des grands et non l'inverse; ou encore: En biologie, la valeur d'un corps ne se mesure pas à son taux! (Quinton)

Avant qu la science ait mis ces idées en relief, la pratique les avait déjà sanctionées à la suite de simples observations.

Ainsi, il y a déjà, long temps que les éleveurs alimentent de façon différente les animaux, suivant qu'ils veulent les mettre en chair ou en graisse.

Pour un même terrain et un même végétal, la chimie agricole moderne a démontré qu'il y a des engrais chimiques qui poussent à la seule végétation et d'autres à la fructification. Enfin, à notre instigation, certains bouilleurs de crû ont noté que si après avoir enlevé à la vendange son alcool et même son acide tartarique, on reportait tout le reste à la vigne, elle n'aurait jamais besoin d'engrais.

. . .

C'est sous l'empire de tout ce qui précède que nous avons projeté de constituer un aliment spécial pour le système dentaire. Pour les raison scientifiques largement développées ci-dessus, cet aliment doit donc contenir *sans aucune exception*, tous les éléments constituants *connus* et inconnus des dents, et autant que possible dans les proportions individuelles déterminées par la nature dans l'organe lui-même.

Pour y arriver, il faudra donc s'adresser aux dents exclusivement comme matière première et choisir celles de l'animal qui, au point de vue omnivore se rapproche le plus de l'homme. C'est pourquoi nous avons pris celles du porc.

[1]Bulletin Soté Chimique 20 Mai 1912 p. 497.

Quand l'animal est dépécé, il est aisé en faisant bouillir ses maxillaires dans l'eau d'en extraire les dents et de les priver ainsi du sable et autres impuretés qu'elles récèlent toujours. L'ébullition au surplus les stérilise une première fois.

Pour ne rien perdre de leurs éléments constituants, il semble alors qu' il n'y a plus qu' à les déssécher, à les pulvériser et arriver enfin à une poudre impalpable d'ingestion facile dans de la confiture.

Mais ces grains de poudre sont d'une telle dureté que leurs aspérités, quoique microscopiques, irritent fortement la gorge et même la muqueuse gastro-intestinale. D'ailleurs, leur texture-pseudo cornée et leur extrême ténacité les rend réfractaires à l'action des humeurs gastriques.

Au contraire, si en cet état on les soumet à l'autoclave de façon assez prolongée, l'osséine se dissout dans l'eau et il devient alors possible de les écraser presque avec les doigts. Mais la division se fait mieux alors avec l'intermède du sucre de telle sorte qu' on peut arriver aisément à un granulé au dixième de dents. Il se conserve indéfiniment.

Il est incontestable, qu'en dehors du sucre, il n'y a ainsi dans le produit rien d'ètranger; que rien de la dent primitive et stérilisée n'a ét perdu, et que les éléments constituants sont bien tels qu'ils étaient dans cette dent, avec leur forme chimique et leur invariable proportion naturelle.

. . .

L'indentité de ce dentogène peut être facilement vérifiées, voici comment: Dans un verre mettons 10 gr. de ce saccharolé et versons par dessus 50 gr. environ d'eau froide. Agitons. Tout le sucre se dissout et le liquide se transforme en bouillie laiteuse. Après un quart d'heure de repos, il s'est divisé en deux parties distinctes; un liquide limpide un dépôt abondant.

Dans le premier, se trouve une albuminoide obéisssant aux réactifs généraux de ces espèces chimiques (a. picrique, tannin, Canres). Mais elle se caractérise surtout comme gélatinoide par son incoagulabilite la par lachaleur, ainsi que par le ferrocyanure acétique et par le nitrate d'argent.

Dans le dépôt on constate la présence des carbonates, phos-

phates fluorures[1] de calcium, magnésium, fer. Tout se dissout à la minute dans quelques gouttes d'acide chlorhydrique. Avec l'acide acétique, la solubilité est ralentie, mais peu à peu il ne reste qu'un minime dépôt qui a bien son importance. Il caractérise, en effect le tissu dentaire lui-même ou plutôt l'enveloppe d'émail fluorurée, reconnaissable au microscope en ce qu'elle affete l'aspect de plaques formées par des prismes verticaux accolés parallèlement. Ce dispositif leur donne le facies d'un carré de toiture de maison.

Un pareil mélange naturel nous parait mériter le nom de *Dentogène scientifique.* Sa constitution exige qu'il soit pris au moment de la grande sécrétion gastrique, c'est à dire au cours des repas.

Les combinaisons phosphorées et fluorurées s'y trouvent à la fois sous la forme minérale et sous la forme organique.

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[1]L'expérience nous a démontré que le fluorure de calcium est très abondant dans toutes les parties de l'invoire de l'éléphant et de l'hippopotame. Nous estimons que c'est surtout à ce fluorure que ces dents doivent la finesse si remarquable de leur grain, leur dureté, leur ténacité.

Dans les dents du porc, on retrouve une part de cette répartition générale et régulière des fluorures, dans certaines grosses molaires parfois très larges chez les individus de grande espèce; mais on ne la retrove plus dans les canines et les incisives. Ici, le flourure parait se concentrer dans les parties superficielles tranchantes et piquantes. On s'en aperçoit vite en désséchant ces dents dans une étuve fortement chauffée. Comme le coefficient de dilatation est différent dans les diverses couches, elles se séparent spontanément à un moment donné. La partie superficielle, celle qui correspond à l'émail, es, beaucoup plus fluorée qu l'autre.

Quoique les défenses du porc soient creuses, elle offrent leur maximum de dureté vers la pointe; c'est là aussi que s'accumule l'émail très fluoruré. Dans les défenses si redoutables du sanglier, il doit exister un dispositif analogue.

Dans les dents humaines, on constate parallèlement que le même fluorure se porte principalement dans les parties directement agissantes. Le but de la nature est ici de leur donner plus de puissance et de dureté au point de vue de la déchirure de l'aliment, de sa mastication et de la résistance de la dent à l'usure. Enfin, le vernis fluoruré ou émail soustrait le corps de la dent à toute intrusion microbienne.

THE ORIGIN AND SIGNIFICANCE OF STARCH

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Introduction

The green leaves of plants possess the most efficient means of transforming the radiant energy of sun-light into the potential energy of carbohydrates like starch, cellulose and sugars. This process which is of fundamental importance to both plants and animals, is carried on by the chloroplasts or chlorophyll-bearing granules of the plant cell. Drawing upon the sun as their source of energy plants are the producers of a form of energy stored in carbohydrates while animals dissipate this energy in the functions of their bodies. They are spendthrifts, too, and were it not for the equilibrium maintained between these opposed functions in animals and plants the world would long since have become bankrupt for the energy so necessary for the existence of living organisms and human industries. These chloroplasts of plants provide the means of absorbing and storing for later use the incalculable amounts of radiant energy poured down upon us daily by the sun. The cellulose present to such a great extent in all living vegetation, and also in the carbonized plant remains in coal, represents one type of energy stored in carbohydrates. In starch we have another example except that it does not form such a permanent reserve for it is consumed relatively soon either by the plant producing it or by an animal. It is this *active* role of starch in its biological relations that makes it interesting. The origin of starch in the plant has offered an attractive field of study that has been worked very industriously for a long time. The biological significance of starch is something that appeals strongly to the biochemist. The industries in which starch figures are great ones and worthy of the closest study but in this place we are concerned with starch in its early history long before it has entered into the food and industries of the people.

Early Theories of Starch Formation

Before we can obtain any idea of the mechanism of the green leaf in its role of starch former we must consider the point of view of the early investigators. First of all, however, it is desirable to define the term *photosynthesis* which is used by many plant physiologists and will often appear in this paper. By photosynthesis we mean the action of the green plant in *using the radiant energy from the sun to effect the union of carbon dioxid and water thus producing gaseous oxygen and sugars* which subsequently may appear as carbohydrates or may be changed into the fats and proteins of the plant. This phenomenon has been and is still sometimes called carbon assimilation. The latter term expresses the idea correctly but does not make enough differentiation between the action of the green plant which *manufactures* its own carbon compounds and that of the lower plants and animals which can only use such compounds in assimilation after they have been elaborated elsewhere. Furthermore, the word photosynthesis clearly expresses the idea that *light* is the fundamental fact in this type of assimilation.

Priestly, to whom the chemists owe so much, found that green plants would grow in confined air rendered irrespirable by the combustion of a candle or exhalations of an animal. He said:

"Accordingly on the 17th of August, 1771, I put a sprig of mint into a quantity of air, in which a wax candle had burned out, and found that, on the 27th of the same month, another candle burned perfectly well in it. This experiment I repeated, without the least variation in the event, not less than eight or ten times in the remainder of the summer."

Later, in 1779, Ingenhoues showed that this purification of bad air by growing plants could take place *only in the light.* Next Senebier proved in 1782 that the carbon dioxid in water, in the soil humus, etc., was far too slight in amount to supply the needs of the plant and that the *atmospheric* carbon dioxid was the source of carbon dioxid for the plant. Lavoisier overthrew the phlogiston theory in which Priestly and the others believed. His methods of exact quantitative study were followed by Saus-

sure who announced in 1804 that there were definite quantitative relations existing between the intake of carbon dioxid, output of oxygen, etc. This bare outline of the early history of the study of photosynthesis will serve as an introduction to the later work which will now be treated under several heads in order to keep a clear outline before the reader.

The Formation of Starch

Decomposition of Carbon Dioxid. When an aquatic plant is illuminated the most obvious result of photosynthesis is the appearance of bubbles of gas. Upon chemical examination this gas proves to be nearly pure oxygen. By counting the number of bubbles produced in a given time one may estimate roughly the rate of photosynthetic action. By exact measurement in eudiometer tubes it is found that for every volume of carbon dioxid absorbed an *equal* volume of oxygen is set free. This is an important observation and will be referred to later. Such plants when submerged in dilute solutions of reduced dyes or venous blood cause the color changes characteristic of oxidation. The so-called bacterium method of Englemann offers a most striking means of demonstrating the production of oxygen when green plants are exposed to light. He used an air-tight preparation of a living green alga surrounded by certain bacteria which are strongly attracted by oxygen but are motionless in its absence. Now, when such a preparation is illuminated these bacteria immediately become active and all move to the centers of oxygen production which are *only* those cells in the *light.* In the darkness and in the presence of light of wave-lengths too short or too long to be visible to us the amount of oxygen set free is very small and consequently the bacteria are motionless.

The power to decompose carbon dixoid into oxygen and to build up sugars seems to be localized in the chloroplasts or green granules of the cell. For photosynthesis to go on it is necessary that we have the following intact mechanism in the leaf: the living chloroplast, a sufficient supply of carbon dioxid, light of the proper wave-length, the proper temperature and an adequate supply of water. The latter is usually ample because the evaporation from the leaves create a constant transpiration current

of water from the roots where it is absorbed through the stem to the leaf. The supply of carbon dioxid comes from the atmosphere where it is constantly present to the extent of 3 or 4 parts per 10,000. This seems to be a very small working capital but when we consider the easy access to the interior of the leaf through the multitude of little openings or stomata one realizes that while photosynthesis is taking place the internal leaf structure is a *vacuum* as far as carbon dioxid is concerned, and so the atmospheric store of this gas is ample for the purposes of the plant. However, it should be stated that an increase of carbon dioxid to ten times its ordinary amount seems to be used by the green plant to good advantage. Millions of tons of that gas are poured into the atmosphere by the respiration of all living things, the decomposition of organic matter by micro-organisms, and the combustion of fuel in the furnaces of industries and homes yet the balance is maintained by the green vegetation of the earth which decomposes this carbon dioxid to build up enormous amounts of organic matter, renewing the air at the same time with the life-giving oxygen. The water and air currents flow this way and that, thus helping in mixing and transporting the gases and keeping conditions uniform for plants both on land and in the water. In Carboniferous times green plants were in their glory because the conditions of high temperature, high content of carbon dioxid in the atmosphere and an abundant supply of water allowed them to reach an unequalled period of activity, the story of which can be read to-day in the world's coal mines.

Role of Chlorophyll. Besides the undoubtedly esthetic part played by chlorophyll in clothing the earth's vegetation with its restful green color it also plays a necessary part as the active agent in photosynthesis. In the chloroplasts this green coloring matter exists either in the form of a thin skin over the protoplasm or in granules within it. The chlorophyll may be extracted with alcohol to give a dark green solution having a beautiful red fluorescence in reflected light. Such an alcoholic solution when shaken with benzene yields a yellow alcoholic layer and benzene soluble fraction having a blue green color. The yellow substance is mostly carotene hydrocarbon crystalizing in orange plates and having the empirical formula C_{40}, H_{56}. The blue green frac-

tion has a much more complex nature and is a mixture of the so-called "chlorophyll" or cyanophyll with other closely related substances. The photosynthesic activity is associated with the blue green pigment and consequently much study has been given to it. It may be obtained in a crystalline form but probably in an altered condition. Many formulae have been given it; some investigators claiming that it contains nitrogen and phosphorus (a lecithin-like substance), and others that it contains a high percentage of magnesium.

The literature of chlorophyll is voluminous and investigators like Willstaetter, Machlewski, Stoklasa and others have all carried on series of researches upon it. Among the decomposition products of chlorophyll there are found substances nearly identical with those from haemoglobin, which is as essential for the continuance of the life of higher animals as chlorophyll is for the green plants. Any detailed discussion of the chemistry of chlorophyll would be out of place here but for many it is a fascinating chapter in modern organic chemistry.

Action of Sunlight. An alcoholic solution of chlorophyll shows a striking absorption band in the red which corresponds to wavelengths of about 640 to 670 microns. Experiments with spectra thrown on living leaves show that it is in just this region of the spectrum that the greatest formation of starch takes place. So, then, it is the energy absorbed from this region that carries on the photosynthetic transformations. The energy thus absorbed is largely turned into heat which always raises the temperature of the leaf and consequently only a small fraction of the absorbed energy is ever converted into the potential energy of carbohydrates etc. On a bright summer day when we absorb certain light rays with our skin the energy thus converted soon causes the well known unpleasant effects, and likewise when this action takes place on a photographic plate the sensitive silver salts are altered in such a manner that a permanent record of any scene may be produced at will. Some think that chlorophyll acts as a sensitizer in photosynthesis just as certain fluorescent substances do in other photochemical reactions. Others look upon the role of chlorophyll as being that of aiding in the transformation of radiant into electrical energy which then splits the carbon dioxid and water into the first products of photosynthesis.

The amount of light required for photosynthesis is not great and so upon exposure to weak illumination the process of carbon dioxid decomposition begins at once but may not become evident since the evolution of oxygen does not occur until the amount set free is in *excess* of that required for the processes of respiration. It is likely that in most conditions under which plants exist the limiting factor in photosynthesis is not lack of light but absence of sufficient carbon dioxid, water or favorable temperatures. Certain shade-loving plants thrive in a very dim illumination but in such cases the cells containing the chloroplasts are often arranged like lenses to focus the available light upon the chloroplasts. In ordinary plants the cells have many ingenious ways of focussing light upon the chloroplasts and of securing favorable alignments by means of changes of position of the chloroplasts in relation to the incident light. On a larger scale, we notice, that each leaf tries to secure the most favorable arrangement for itself, an arrangement resulting in the least shading of the leaf by others. This tendency produces " leaf mosaics " of great interest and beauty. Many plants when viewed from above (whence the most light comes) present a nearly unbroken expanse of green leaves thus enabling the plant to make the most of all the light it does receive. The plant even in strong light does not begin to form starch at once when illuminated but only after the lapse of a certain time during which, apparently, the precursor of starch has collected in sufficient quantity to start the mechanism of starch formation. The increase of dry weight of an illuminated leaf does not represent the total amount of products formed but only the quantity *remaining* in the leaf, the rest of the material produced having been translocated in diffusible form to another organ of the plant where it is laid down in the form of the so-called " secondary starch " as in potato tubers.

Nature of Photosynthetic Products. We have already seen that the volume of carbon dioxid absorbed and oxygen disengaged are nearly equal and, further, that the first distinguishable substance is starch. Now, starch has a very high molecular weight, variously estimated at from 12,000 to 30,000, and it does not seem probable that such a complicated substance should be produced at once from water and carbon dioxid. Baeyer's theory that formaldehyde is first produced and that it soon condenses to form sugars

is well known and it probably expresses correctly the nature of photosynthesis. It has been generally accepted that glucose is the *first stable* product from which starch, sugars, fats, and proteins may be constructed according to the needs of the organism. In most plants during the day this glucose is rapidly condensed to starch which fills the cells but as evening and darkness approach photosynthesis is retarded and the starch is converted back to glucose and similar easily diffusible substances which are easily translocated to other parts of the plant. Assuming that glucose is the first stable product we may write the reaction for photosynthesis as follows:

$$6\ CO_2 + 6\ H_2O = C_6H_{12}O_6 + 6\ O_2.$$

This equation, however, does not represent the whole truth but indicates only the general trend of transformation, the important but unknown intermediate products as well as the energy relations being ignored. The heat of combustion of glucose is about 3.75 Calories and all of this energy must have come from the sun in the beginning.

It is by no means true that all plants store energy in the form of starch although many of them do so. In certain groups of plants such as the lily, orchid and amaryllis families very little if any starch is formed while in the legumes and Solanaceae large quantities are present. When starch is not produced we find substitutes in the form of cane-sugar in several plants, mannite in the Oleaceae, etc. The oils, proteins, glucosides and so on are probably not the direct result of photosynthesis but are produced later by the union of glucose with other substances or by condensation with itself to form more complex carbohydrates. The first substances produced by photosynthesis are extremely active chemically and it may well be that, at this stage and in the presence of nitrates, phosphates, and sulphates the proteins are constructed. In the green leaf many optically active substances are formed, a type of synthesis difficult to perform in the laboratory without the intervention of the experimenter or other living organism able to diffierentiate between the right and left handed modifications.

In darkness, even in the absence of chlorophyll, the plant cells can store up starch if fed with glucose, sucrose, glycerine and

many other similar substances. This shows that the photosynthetic and starch forming processes are distinct. Proteins, fats and many other types of organic materials may all be formed in darkness also. Some observers have reported that in the light the chloroplasts of certain algae seem to show a shrinking and change of their protein substance into starch. It may be that one step in photosynthesis is the disintegration of the protein of the chloroplasts to split off carbohydrate in this manner.

Artificial Photosynthesis. The idea that formaldehyde is an intermediate product of photosynthetic activity has led many investigators to see first if it really may be detected in green leaves by chemical means and secondly if it may be made to condense and produce sugars artificially. Several investigators have found that leaves do give a positive test for formaldehyde but whether formaldehyde *itself* were present can not be said. A more complex aldehyde has recently been isolated by Curtius and Franzen from certain leaves. It possesses the six carbon atom skeleton characteristic of glucose. Attempts to cause starch formation by feeding formaldehyde or its derivatives to plants have been partially successful. It is interesting that in alkaline solutions formaldehyde condenses with itself to give a sugar like glucose. Under certain conditions the silent electrical discharge breaks up carbon dioxid into formaldehyde which, in turn, may then be converted into sugars. In the presence of alkalies Stoklasa found that ultra-violet light changed a mixture of carbon dioxid and nascent hydrogen into sugars. When formaldehyde and oxalic acid were sealed in glass tubes and exposed to sunlight, those tubes only which were thus exposed were shown to contain considerable quantities of sorbose. The action of light and of the traces of alkali in the glass seemed to catalyze this reaction. Electricity and ultra-violet light seem to lower the temperature necessary for these condensations to take place. Experiments of a different type have been carried out in which a thin film of chlorophyll was deposited on water or gelatine and then this artificial leaf was illuminated and a little catalase added to decompose any hydrogen peroxide formed. Under conditions of illumination and presence of carbon dioxid the experimenters reported the formation of small quantities of formaldehyde.

All of these recent investigations show that the formaldehyde theory of sugar and starch formation has experimental ground for its existence and, at any rate, it is helpful in visualizing some of the processes of photosynthesis. Such observations also force us to consider that, after all, photosynthesis is not wholly a vital process but that under the proper conditions it may be imitated in the laboratory though in an inefficient manner.

Physical Nature of Starch

Ordinarily we see starch in the form of a white powder which gives a peculiar rustling sound when rubbed between the fingers. Under the microscope the whole appearance changes and the starch grain now takes on a characteristic form depending upon the organ and species of plant from which it came. This form is nearly constant for any given type of starch. The size of the grains varies from the large one of the Canna (visible to the naked eye) to the most minute sort. The form of the larger types like the starch from potatoes may best be described by likening them to oyster shells often with eccentric striations. In the case of corn and rice starch we do not have a simple grain but a compound structure consisting of many small grains having more or less angular faces. In polarized light the familiar black cross appears and this shows that the starch grain has a definitely organized structure of some sort.

The effect of starch on polarized light and its peculiar striated or stratified appearance have led to the publication of many theories to explain its internal structure. The layers may probably be accounted for by assuming that they represent the product of varying periods of activity on the part of the functioning chloroplasts or leucoplasts. When starch is formed in the green leaf it is produced on the chloroplast of its origin while in tubers and other storage parts it is made from glucose and maltose by the activity of the leucoplasts or colorless granules which are seats of this storing action. The layers and striations of the grain are seldom concentric because the centers of starch formation are usually not the geometrical center of these protoplasmic granules. The latter are often far smaller than the starch grain grow-

ing upon them. It must be remembered that the formation of starch from the products of photosynthesis by either the chloroplasts or leucoplasts has little to do with the photosynthetic fuction of the former but is controlled by the amount of glucose and maltose in circulation in the plant. Some authors consider that the different layers are caused by variations in the water content of the starch deposited. It was also thought for a long time that the outer envelope of the starch grain was a cellulose because of the well known insolubility of starch in cold water and the difficulty in digesting raw starch by enzyme action. The true starch or amylose was supposed to be in the interior and to imbibe water through the cellulose envelope; this causing a swelling which ruptured the envelope, yielding the familiar starch paste. Arthur Meyer believed starch was composed of sphaero-crystals consisting, in turn, of radiating needle-like crystals of two sorts, one easily soluble in water and giving a blue color with iodine and the other a substance less soluble in water like the cellulose envelope of the earlier writers. The conception of the starch grain as a sphaero-crystal is interesting and there is some experimental evidence for it. At present it is impossible to state with certainty that starch has one type of structure or the other.

The Chemical Nature of Starch

We have just seen that starch is apparently composed of two substances, one of which is water soluble and possesses all the properties commonly associated with starch while the other is more insoluble and more like cellulose in its behavior. Treatment with boiling water, acids, alkalies and digestive ferments gives first a thick colloidal solution having well marked starch reactions which decrease in intensity and finally give place to simple solutions and more active chemical properties as hydrolysis into dextrins and sugars progresses. Soluble starch is the first hydrolytic product but it is soon changed into the dextrine. The chief characteristic of soluble starch is that it dissolves in warm water to give a clear solution having the usual starch properties unchanged. This form of starch may be made by treatment with very dilute acids, alkalies, or by enzyme action, pro-

vided, of course, the reaction is arrested at the proper point. A great many interesting and industrially important starch derivatives are manufactured but they are too numerous to mention here.

The blue coloration with iodine is the commonest means of detecting starch and it is a striking and valuable test. Much study has been given it but we still lack accurate information about it. Some consider starch iodid a chemical compound, others an absorption phenomenon and still others think of it as a solid solution of iodin in the colloidal contents of the starch grain. The blue color is easily destroyed by heat but reappears on cooling and, furthermore, it is very easily changed by numerous chemicals. Not all starches stain a pure blue with iodin; some give purple and some even give red colors. This probably indicates a difference in the complexity of starches from different sources. With iodin a shade of red or brown indicates a departure from natural starch and an approach to the simpler dextrins and, finally, to the simplest and well known sugars. During digestion by diastase the starch grain is corroded and attacked more in certain portions than in others. This fact may indicate a difference in chemical nature between the different layers of the grain as already suggested.

In the classification of the carbohydrates starch is listed as a polysaccharid and it is from this word *poly* that we get the key to the whole matter. We ought to consider starch as being built of many glucose and maltose units connected in such a way that no carbonyl groups are free. This we know because, like saccharose, starch shows none of the reactions characteristic of such a group. The usual formula for starch is $(C_6H_{10}O_5)n$ in which n may be any number from 20 to 200. It is almost impossible to obtain accurate data on the molecular weight of starch but from physico-chemical studies, chemical derivatives and ultra-microscopic observations it seems likely that its molecular weight may be from 10,000 to 30,000, figures probably not often equalled even by the complex proteins. In the plant the processes of building up this complicated molecule and of breaking it down seem to be reversible and are probably under the control of enzymes. Apparently the active mass of the glucose and maltose in the food-

conducting system of the plant determines the course of this reversible reaction and determines whether its direction shall be towards the storing of starch or towards its hydrolysis into the more diffusible and immediately available sugars. The complexity of starches from different sources is a variable factor and so by starch we can only mean a general term including those substances having most of the reactions and properties commonly associated with the well known starches of commerce. More exact studies upon the chemistry of starch with the improved methods of the recent advances in chemistry ought to yield the most interesting and valuable results.

Significance of Starch in the Plant

In the earlier chapters it has already become evident that starch acts primarily as an indiffusible but easily convertible form of stored energy. The heat of combustion of starch (4.1 Cal.) is slightly higher than that of glucose but as a form of potential energy it cannot compare with the fats and oils which have an energy value of about 9 Cal. However, in many plants starch is the most abundant form of stored food and is, possibly, more easily converted into its constituents for purposes of translocation than are the fats. The proteins are more likely to appear as integral parts of the living protoplasm than to act as stores. Most of the starches with which we are familiar are nearly always prepared from some storage organ of the plant and have larger and better characterized grains than the primary starches in the leaf. The leucoplasts of the fruit pulp, tubers, etc., of the plant are the active agents in reforming starch from the translocation stream of sugars. There is a form of starch storage in which the leucoplasts do not seem to play any part. The type is represented by the somewhat temporary starch reservoirs found in pollen grains, the sheath of growing tissue, and so on. Under such conditions the starch exists in a very finely divided state and appears to be a store of a transient nature. In either form of storage the enzyme diastase seems to cause the transformation of starch into its sugar constituents and also the reverse change when circumstances demand it. The so-called translocation diastase of the

green leaf causes the change there while the secretion diastase of germinating seeds and tubers carries on a similar action in those places. The two sorts of diastase do not corrode the starch grains in the same manner nor are their other properties exactly the same. Although starch is laid up in enormous quantities in the tubers, seeds, stems and pulp of fruits, it is far from being the only polysaccharid thus stored. Glycogen has the same function in the fungi and so has inulin among plants of the Compositaceae and Liliaceae; sucrose acts likewise in sugar-cane and beets, while glucose is found in the leaves and bulb of the onion. However, starch and cellulose are the two great stores of energy in the form of carbon compounds that are produced so abundantly by nature each season.

Significance of Starch to Man and Animal

In the early history of the race our ancestors probably noticed that certain animals and birds sought much of their food in the seeds of grasses while at the same time the smaller animals dug into the earth for roots and tubers. Thus man early learned to make the starchy foods one of the main articles of his daily fare and it is true to-day that among all peoples in all climates bread from cereals or some starchy substitute is the "staff of life." Among many animals the foods of this type are the staple ration and it is only the carnivora that scorn such a diet. Upon digestion the starches are split into the sugars which are then burned in the organism to yield their energy for the maintenance of the physical activities and physiological functions of the animals. Unlike the proteins, the carbohydrates and fats are used by animals to produce heat and energy and not so much to become living protoplasm as is the case with nucleo-proteins and albumins for example. Since but little new protein is needed for the upkeep and growth of the mature plant or animal we see that the constant demands for energy supplies must be met by the sugars and fats consumed. The abundance of starchy foods eaten by men and animals is adapted to meet this necessity of energy producing material in large quantities.

The greatest source of starchy food is, of course, the seeds of the various cereals which we group together as grain. The amount of

such material produced from the soil in a year is almost beyond calculation. The production of this golden flood of grain is the earth's oldest and greatest industry. Besides the starch given us in the cereals we must not forget the potato which is another staple article of diet in the whole civilized world. In different countries various starchy foods are popular such as sweet potatoes, arrow-root preparations, tapioca, sago, chestnuts, bananas, etc. From the time that man first noticed that grains were good to eat he has taken plants of this type under his special protection and given them careful cultivation. The result has been an improvement in the races of grains as judged by their yield and adaptability to varying conditions of climate. To produce these harvests the soil supplies the water and mineral nutriments while the carbon dioxid and sun-light lend their aid through no effort of man. His duty, then, is to see that the soil is kept in its most productive condition and by so doing he will have an ample supply of grain for the needs of the future.

The Industrial Importance of Starch

The observation of primitive man that the seeds of certain plants made an acceptable food was the beginning of agriculture. Another observation made sometime later was that when starchy materials were allowed to stand they underwent a peculiar transformation. The result of this change was a so-called "spirit" which was soon found to possess magic properties in making "glad the heart of man." This, then, was the origin of another vast industry whose object is the production of alcoholic materials through the fermentation of grains by enzymes and micro-organisms. Alcoholic beverages of one sort or another are known everywhere and their production goes hand in hand with the practice of agriculture. The amount of grain used by the brewing and liquor distilling industries comes to an enormous figure and is second only to that consumed as bread and various bakery products. The flour milling industries prepare starchy food for the millions, the example of the former in centralization is being followed more and more by the bakeries, especially in the larger cities. The preparation of bread in the home is becoming

less common every year and most of this work is done in large bakeries where more or less scientific methods are beginning to prevail. Various forms of natural and prepared starch are employed in large quantities in the form of specially treated foods, laundry starch, sizings, adhesive pastes and so on in great variety. Very valuable products are manufactured by heating or treating raw starch in such a way that dextrins and gums are formed. These are used as adhesives and for other purposes. The action of dilute acid upon starch yields glucose and it is upon this reaction that another great industry has been founded. Glucose has a multitude of industrial applications and it also figures in our food, sometimes under another name but tasting just as sweet. Starch and its products are valuable in many other ways than merely those already mentioned but it would be presumptuous to point them out to this Section of our Congress.

In this paper the writer has not striven to give detailed discussions of any sort for these may be found in books on plant and animal physiology but has endeavored to present many old and a few new ideas in the way that they appear to one interested in the biochemical problems of plants and animals. For those desiring a closer insight into the phenomena of starch formation a short bibliography is appended. In these works full references to the original papers in this field may be obtained.

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INFLUENCE DES IMPURETÉS GAZEUSES DE L'AIR SUR LA VITALITÉ DES MICROBES

Par M. A. Crillat

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L'air que nous respirons contient des impuretés gazeuses variées. Jusqu'ici, on n'avait pas songé à étudier leur influence sur la vitalité des microbes en suspension dans l'atmosphère. Au point de vue épidémiologique, cette étude, qui peut servir à expliquer le mécanisme encore si obscur de la contagion par les germes de l'air, présente un grand intérêt.

Les expériences ont tout d'abord démontré que les microbes, dans l'état spécial où ils se trouvent dans l'air, sont extraordinairement sensibles aux moindres variations de la composition chimique de l'atmosphère. Les influences sont tantôt antiseptiques, tantôt activantes ou conservatrices, et j'ai désigné sous le nom d'ambiances favorables l'air contenant des gaz susceptibles de prolonger l'existence des microbes ou de faciliter leur reproduction. L'analyse de ces gaz a déjà démontré qu'ils renfermaient des substances alcalines gazeuses, parmi lesquelles on a distingué à côté de l'ammoniaque des amines et peut-être des alcaloides gazeux. Les ambiances favorisantes se produisent dans une foule de cas: dans la décomposition putride des substances animales ou végétales, dans le voisinage des matières fécales, dans les émanations du sol, dans l'air souillé par la respiration humaine, etc. Toutefois, la présence de ces gaz dans l'atmosphère ne suffit pas pour constituer une ambiance favorable; il faut le concours d'autres circonstances que j'ai étudiées.

D'après mes travaux, les altérations subites des matières alimentaires sont intimement liées à la formation des ambiances favorables, et j'ai vérifié cette hypothèse par une étude des causes de l'altération du lait pendant les temps d'orages.

La présence de ces gaz à l'état de dissolution dans l'eau lui communique la propriété d'être très favorable au développement

du bacille typhique. Enfin, leur introduction dans l'organisme des animaux exalte la réceptivité vis-à-vis les germes pathogènes.

L'ensemble de ces résultats obtenus montre donc l'importance de cette étude à plusieurs points de vue.

EXPERIENCES WITH DUODENAL AND STOOL FERMENTS IN HEALTH AND DISEASE

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Interest of recent years has centered on the attempt to diagnose abnormal conditions of the pancreatic gland and its secretion by testing the contents of the gastro-intestinal canal or the urine for pancreatic ferments. The stool and urine have been mainly utilized; of late years also the stomach contents after an olive-oil test meal.

It is apparent that these methods are indirect ones, involving irregular dilution of the elaborated enzymes with other body fluids.

The suggestion of both Einhorn and Hemmeter to use a tube which shall enter the duodenum offers a direct method for obtaining the external secretion of the pancreas as elaborated. Duodenal contents so collected were examined and a comparison made with the ferment analyses of the stool in the same cases.

Method. The Einhorn Duodenal Pump was utilized. It consists of a long thin rubber tubing and an attached perforated metallic capsule. The patient swallows the capsule and attached catheter to a point on the catheter marked 80 centimeters. This was done at night; in the morning two and one half hours after the patient had drunk eight ounces of milk, the duodenal content was aspirated for five minutes.

The material obtained was judged to be duodenal contents when either a radiograph showed the metallic capsule in situ or a distinct " retraction test " was noted. When the capsule occupies the duodenum the material enters the aspirator slowly; a resistance (the walls of the intestine) is felt. When the capsule, on withdrawing, enters the stomach there is a rapid gush of material, usually of an entirely different nature. This is the " retraction test."

The duodenal juice is usually golden yellow, viscid, slightly acid (due to gastric contents) or neutral; in amount ten to forty cubic centimeters. The stomach content is milky white and strongly acid.

The contents of the duodenum as obtained were diluted with twice as much distilled water; a part was immediately made slightly alkaline with sodium hydrate solution, this serving for alkaline protease test; the remainder was used for testing amylase and lipase.

The chemical methods for analzying the duodenal ferments were as follows:

For Amylase: One cubic centimeter of the duodenal juice was tested against increasing amounts,(½ to 6 cubic centimeters) of 1% soluble starch solution, the volume in each test tube being made up to 10 cubic centimeters with water. The incubation time was one hour and the persistence of starch tested for by adding a small excess of Lugol's solution. The last tube to show disappearance of starch was read and the number of cubic centimeters of starch solution used, multiplied by the dilution, was accepted as the factor.

In the earlier tests the Wohlgemuth method was employed. The method was discarded because of the inconstant results obtained by testing with only one to two drops of iodine solution.

For Lipase: To ten cubic centimeters of distilled water were added one cubic centimeter of the material to be tested, one cubic centimeter of ethyl butyrate, one cubic centimeter of toluol and a drop of phenolphthalein solution; the whole made up to 25 cubic centimeters and neutralized. After shaking forcefully for fifteen seconds, it was again brought to the neutral point. A control test was always prepared with boiled duodenal contents. After incubation for 24 hours, both flasks were titrated and the amount of acid in the control subtracted from that in the test flask, and the result multiplied by the dilution.

For Protease (alkali): Mett tubes, cubes of coagulated egg albumin, Fermi gelatin tubes and the Gross-Fuld casein method were utilized.

In the stool, amylase was estimated by the Wohlgemuth-Hawk method in a slightly different form. Here again iodine was added in excess to test for the persistence of starch.

Lipase and protease tests were the same as in the duodenal tests. For all the stool analyses, a dilution of four parts of stool to fifty parts of slightly alkaline water was used. Usually no catharsis was used in obtaining the stool.

Technical Discussion of the Tests: In all the instances, the fluid was removed in the morning and immediately iced until examined in the afternoon. The acid reaction was preferable for preserving the fluid for both amylase and lipase tests; in acid reaction these ferments could be preserved for 24 - 48 hours in undiminished strength. In alkaline reaction an apparent antodigestion took place very rapidly, probably due to the presence of trypsin.

The point was frequently raised as to whether the amylase test obtained was due to the salivary or due to pancreatic enzyme. Numerous tests of the stomach contents in these same cases showed the absence of a ferment capable, after five hours' maintenance in an acid reaction, of digesting starch. The duodenal contents, though always containing some of the same acid gastric material, rarely failed to show an active amylase; it is probable therefore that pancreatic amylase is unaffected by pepsin; salivary amylase destroyed by it.

Lipase was similarly best maintained in faintly acid medium; it was destroyed in part or totally in an alkaline medium containing other active pancreatic ferments.

Trypsin was always found in its activated state. This enzyme was best maintained in an alkaline medium.

The duodenum normally contains at least two proteases, trypsin and erepsin, the latter secreted by the duodenal mucosa as well as by the pancreas. Of the tests utilized for demonstrating the proteases, neither the Mett tubes, coagulated egg albumin cubes nor the Fermi gelatin tubes are attacked by erepsin. Casein is digested by erepsin, but in a series of experiments concentrated extracts of the duodenal and intestinal mucosa of the dog, cat, and of man digested casein in dilutions of only 1 : 10 to 1 : 140 while the active pancreatic secretion digests the same amount of casein in dilutions up to 1 : 200,000. We may conclude that though the erepsin is present, its faint proteolytic action on casein does not really affect the value of the figures

obtained, we are safe in interpreting the result as truly tryptic activity.

A similar interpretation may be held for the results of stool analyses. Where slight digestion took place in strong dilutions, the result may have been due to erepsin; where the proteolysis is complete in the much higher dilutions, the result may be interpreted as due to trypsin. Frand and Schittenhelm assert, on the basis of differential polypeptic-splitting tests, that the protease of the stool is usually erepsin and not trypsin. It is difficult to harmonize the above facts with this assertion. Further, stools which actively proteolyzed casein frequently also liquified gelatin; this could be due to trypsin only.

Results of Tests of the Duodenal Contents of a Normal Person

A male adult furnished repeated specimens.

See Table I

From a study of this table, it will readily be seen that quantitative estimates of the strength of pancreatic ferments obtained from the duodenum of a normal man vary within wide limits. In practically every instance, the three ferments tested for are found in an active state. Lipase was absent on one occasion.

Results of Tests in Cases of Interest Because of Pathological Conditions :

See Table II

The cases observed are discussed in groups.

Group A. This comprises one case of acute pancreatitis with a diffuse abscess involving the head and tail of the organ. In the duodenal contents the ferments are absent except for lipase, which is feebly present. Examination of the stool demonstrated the same conditions as in the duodenum.

Group B. Cases of Cholelithiasis (Gall-stone Disease): The ferments are here found in an active state in the duodenum. A wide range of variation is observed, yet in general the ferments are either normal or hypernormal in their activity. In case 6 the absence of amylase and lipase suggested a diseased pancreas. At operation the head of this organ was found swollen and edematous to a marked degree.

Group C. Cases of Obstructive Jaundice: The point of interest was, Is the pancreatic duct open and the pancreas secreting? In the first two instances (cases 7 and 8) this duct was evidently open and active pancreatic ferments entering the intestine.

In case 9, on first examination, the absence of all the ferments from the duodenum except a weak lipolytic ferment, and the absence of all the ferments from the stool led to the diagnosis of complete pancreatic obstruction involving all the ducts possibly emanating from this gland. On a second examination, several weeks later, the results of both duodenal and stool analyses indicated some return of pancreatic ferments in the intestine. At autopsy the head of the pancreas and the duodenum were found involved in a massive sarcomatous tumor; the ducts back of the new growth were greatly dilated and distended with fluid. It seems probable that from time to time the pressure in the ducts was sufficiently great to force pancreatic secretion through the new growth and into the intestine.

Group D represents the findings in two cases of hypertrophic cirrhosis of the liver. From the ferment analyses, the pancreas would seem to be secreting fluid of high potentiality. The examination of the stool in the one case agrees with the findings in the duodenal material.

Groups E and F. The ferments are present and active except for amylase, which is absent in two instances. The failure to demonstrate this ferment was probably due to faulty technic in the early tests.

Group G. In this group is collected the data for various gastric diseases. As far as one can judge, there is no evidence of disturbance of pancreatic secretion. It is of interest to note the absence of trypsin in the case of carcinoma of the stomach.

Case 21, one of achylia gastrica, requires a note. Repeated analyses of gastric contents showed the absence of both pepsin and rennin as well as all trace of acid. The pancreatic secretion is, however, active, all the ferments being present. Ehrman and Lederer, employing the Volhard test meal, found active pancreatic ferments in these cases. In the duodenal contents, however, obtained by me in this case, no rennin was demonstrable. It is still a question whether the human pancreatic gland secretes a ferment capable of coagulating milk.

Group H. Cases of Diabetes Mellitus: In general it may be said that these cases do not show any variation from normal figures, all the three ferments tested for being found present and active. In only one instance was the reaction for amylase weak (case 25). In the instance of case 26, on a strictly limited milk diet, the ferments were all only feebly present. A later examination, on a more full diet (oatmeal and milk) gave ferments of greater strength.

Case 27 was of interest, being a case of diabetes mellitus in a female adult with a distinct history of cholelithiasis and abdominal attacks indicating pancreatitis. The stools in this case were bulky and grayish-white and frequent. Metabolism studies indicated even on a restricted diet a loss in the stools of 54% of the fat, and 29.4% of the nitrogen intake, corroborating, so far as our studies of metabolism would indicate, pancreatic insufficiency. The duodenal findings in this case demonstrated a very scant secretion into the intestine, though a secretion of high potentiality. The stool in the same case showed active ferments though only weak protease.

REMARKS. The original intention of this study was to determine: 1, the limits of ferment activity in the normal duodenum; 2, possible variations from these normal limits in pathological cases; 3, to determine in how far the analysis of the ferments of the stool gave an indication of pancreatic activity.

In regard to establishing the strength of the ferments as normally secreted, Table I represents the limits of variations. It would seem that the pancreatic ferments show fluctuations of strength from day to day even under identical conditions, but that such fluctuations may be said to be within limits. However, the occasional failure to detect amylase or lipase in seemingly normal secretions must be noted. These ferments show the greatest variability in strength, and may apparently be occasionally absent. The protease is the most constant and is always present. In spite of the arguments against casein as a test of trypsin alone, I would hold that erepsin though present, is never sufficiently strong to interfere with the test as an index of pancreatic trypsin.

2. Of the pathological cases examined, the case of acute pancreatitis shows decided diminution in the activity of the pan-

creatic ferments. The pancreatic gland was found decidedly diseased at autopsy. In case 9 the ferments were absent from the duodenum on one occasion; also absent from the stool. The diagnosis of complete blocking of the ducts was confirmed at autopsy. In case 6 on account of the absence of two of the ferments from the duodenum, a deficient secretion of the pancreas was expected, though the duct was apparently open. At operation a patent duct, but a large swollen inflamed pancreas was palpated.

In case 27 the metabolism studies indicated pancreatic disease. The ferments were found strongly present; the amount of secretion into the duodenum was, however, very scant.

3. The variability of the same strength of the same ferments in the stool is far greater than in the duodenum. The occasional absence of ferment is a more frequent occurrence. In general, where these enzymes are strongly present in the duodenum, they are also demonstrable in the stool. The protease is here no longer reliable as an index of pancreatic trypsin. For in cases 7 and 8 and 25, though a strong reaction for trypsin was obtained in the duodenal contents, this enzyme was not demonstrable in the stool (casein and gelatin tests). However, in case 9 when the protease was absent from the duodenum it was also absent from the stool; and when it reappeared in the former, it also reappeared, though weakly, in the latter. We may conclude that a positive test for trypsin in the stool signifies an open pancreatic duct, a negative test does not necessarily imply that active trypsin is no longer being secreted into the duodenum.

The question is: Can this method of estimating the enzyme strength of duodenal contents be utilized for the diagnosis of pancreatic functional activity? It is certain that it is reliable for ascertaining the patency or non-patency of the pancreatic ducts. More experience with cases of disease in the pancreatic gland is necessary before it will be definitely known whether the method is applicable to the diagnosis of functional activity of this organ. From the few cases in the series offered, I am inclined to believe that this will be accomplished.

The results on the different days are tabulated as follows:

TABLE I

AMYLASE

3/21 1 c.c. duodenal juice hydrolyzes 6 c.c. of 1 % starch solution in 1 hr.
3/28 1 c.c. duodenal juice hydrolyzes 6 cc.
5/13 1 c.c. duodenal juice hydrolyzes 10 c.c.
5/14 1 c.c. duodenal juice hydrolyzes 9 c.c.
5/18 1 c.c. duodenal juice hydrolyzes 24 cc.
5/20 1 c.c. duodenal juice hydrolyzes 30 c.c.

Normal Average = 14.1 c.c.
Normal Limits = 6-30 c.c.

LIPASE

3/21 1 c.c. duodenal contents require 3.9 c.c. N/10 NaOH after 24 hrs.
3/28 1 c.c. duodenal contents require 3.6
5/13 1 c.c. duodenal contents require 0.6*
5/15 1 c.c. duodenal contents require 0.9
5/18 1 c.c. duodenal contents require 1.9
5/26 1 c.c. duodenal contents require 0.9

Normal average = 1.96 c.c.
Normal limits = 0.6 to 3.9 c.c.

*On one occasion no lipase was demonstrable.

ALKALI-PROTEASE

CASEIN TEST

3/21 Duodenal contents in dilution of 1:4000 digests 10 c.c. 0.1% casein sol.
3/28 Duodenal contents in dilution of 1:36,000
5/15 Duodenal contents in dilution of 1:120,000
5/18 Duodenal contents in dilution of 1:5,000
5/20 Duodenal contents in dilution of 1:36,000
5/21 Duodenal contents in dilution of 1:12,000
5/29 Duodenal contents in dilution of 1:12,000

Normal average = 1:32,000
Normal limits = 1:4000 to 1:120,000.

OTHER TESTS

	Fermi 24 hrs.	Gelatin Tubes 48 hrs.	Mett Tubes	Albumin Cubes
3/21	3.5 mm.	6 mm.	2 mm.	Slight rounding
3/28	8 mm.	11 mm.	1 mm.	
5/18				Much digested
5/20	5 mm.	10 mm.	1 mm.	All digested
5/26	8 mm.	14 mm.		
5/29	10 mm.	15 mm.		
	Normal Average 7 mm.	11.2 mm.		
	Normal Limits 3.5-10 mm.	6-15 mm.		

Table II

				Duodenum						Stool			
				Amylase	Lipase	Casein	Fermi	Mett	Cubes	Amylase	Lipase	Casein	Fermi
Group A													
Case 1* Ac. Pancreatitis	10 c.c.	acid	bile	0	1.8	0				0	.2	0	
Group B													
Case 2* Cholelithiasis	7 c.c.	acid	bile	10	0	1:58,000							
Case 3* Cholelithiasis	15 c.c.	acid	bile	12	6.3	1:12,000							
Case 4* Cholelithiasis	35 c.c.	acid	bile	30	10	1:1,000	3	0		0	.1	1:700	
Case 5* Cholelithiasis	10 c.c.	neutral	bile	24	3.6	1:18,000	10	2	+	10	0.25	1:50,000	2
Case 6* Cholelithiasis (Before oper.)	27 c.c.	acid	bile	0	0	1:5,000	10						
Group C													
Case 7 Obstr. Jaundice New Growth	12 c.c.	neutral	0	4	2.4	1:4,000	3	½	+	0	.3	0	0
Case 8* Obstr. Jaundice Stricture	10 c.c.	acid	0	30	0	1:4,000	3 5			½	1.0	0	0
Case 9* Obstr. Jaundice (a)	10 c.c.	acid	0	0	0.5	0		0		0	0	0	0
Duoden. Sarcoma (b) (b) several weeks later	5 c.c.	acid	+	4		1:600	3	0		2	0	1:200	0
Group D													
Case 10* Hypertrophic Cirrhosis of Liver	25 c.c.	neutral	+	27	—	1:18,000	7	0	+	8	1.5	1:3000	0
Case 11 Hypertrophic Cirrhosis of Liver	50 c.c.	acid	+	15	1.0	1:,4000	1	0					
Group E													
Case 12* Retroperitoneal Hodgkin's Dis.	40 c.c.	acid	+	0	5.6	14:,000	8	1	+ +				
Case 13* Abdominal Sarcoma	12 c.c.	neutral	+	4	3.3	1:500	4	1.5		10	.15	1:1700	0

Note: Cases marked * confirmed by operation or autopsy.

TABLE II (Continued)

					Duodenum						Stool			
					Amylase	Lipase	Casein	Fermi	Mett	Cubes	Amylase	Lipase	Casein	Fermi
	GROUP F													
Case 14	Chronic Colitis	12 c.c.	neutral	+	0	0.6	1:12,000							
	GROUP G													
Case 15	Duodenal Ulcer	20 c.c.	acid	+	20	—	1:6,000				++	1.5	1:5000	
Case 16	Gastric Neurosis	—	Alk.	+	+	—	++				0	0	1:5000	
Case 17	Gastric Neurosis	8 c.c.	acid	+	0	0	+				1			
Case 18	Duodenal Ulcer	15 c.c.	acid	+	+++	0	++				5			
Case 19	Duodenal Ulcer (hour-glass)	3½ c.c.	alk.	+	—	2.4	1:1,400	10						
Case 20	Carcinoma Stomach	7 c.c.	acid	+	9	5.4	0		0		0			
Case 21	Achylia Gastrica Simplex	4 c.c.	acid	+	3	8.4	1:4,000	3	½	rennet	6	.1	1:30,000	
	GROUP H													
Case 22	Diabetes	25 c.c.	acid	+	6	4.5	+++		2					
Case 23	Diabetes	9 c.c.	acid	+	9	12.6	1:4,000		0					
Case 24	Diabetes	15 c.c.	acid	+	30	2.7	1:6,000	∫ 3 \ 7	0	+	1	0	1:700	
Case 25	Diabetes	3½ c.c.	acid	+	1½	6.0	1:4,000	2.5		+	8	.4	0	
Case 26	Diabetes ∫ Milk Diet	25 c.c.	acid	+	6	0	1:34	0						
	Diabetes \ Fuller Diet		acid		6	.6	1:4,000	15	1	+				
Case 27	Diabetes	3 c.c.	alk.	+	15	1.2	1:500	∫ 5 \ 11	1	+	2½	3	1:125	

La biophotogénèse réduite à une action zymasique

MECANISME INTIME DE LA PRODUCTION DE LA LUMIERE PHYSIOLOGIQUE: LUCIFERASE, LUCIFERINE, LUCIFERESCEINE

Par M. Raphael Dubois

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Un nombre considérable d'hypothèses a été émis à propos du secret de la merveilleuse production de la lumière par les végétaux et les animaux.

Les désaccords entre les expérimentateurs sont venus souvent, presque toujours même, de ce qu'ils n'ont envisagé que des cas particuliers. C'est ainsi que les anatomistes surtout ont fait jouer chez les insectes, un rôle capital aux trachées que quelques-uns allaient jusqu'à comparer à des tuyaux de forge embrasant le protoplasme!

Ils ne songaient pas, sans doute, que l'immense majorité des êtres lumineux n'ont pas de trachées et que l'oeuf de l'insecte photogène lui-même brille avant même d'avoir été fécondé, d'une luminosité qui lui est propre, comme je l'ai jadis démontré.[1]

C'est pour remédier aux graves inconvénients des études partielles qu'à; la suite de mon ouvrage sur les Elateridés Lumineux[2] j'ai entrepris une étude générale de la question de la Biophotogenese ou production de la lumière par les végétaux et les animaux, j'ai consulté à peu près tous les documents connus et j'ai pu combler expérimentalement ou par l'observation personnelle un grand nombre de lacunes existant dans ce beau chapitre de la physiologie générale.

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[1]De la fonction photogénique chez les oeufs du Lampyre (Bull. Soc. Zool. de France T. XII 1887).

[2]Thèses de la Faculté des Sciences de Paris et Bull. de la Soc. Zool .de France, 1886 (ouvrage couronné par l'Institut de France, grand Prix des Sciences Physiques).

Cette étude d'ensemble a présenté pour moi deux grands avantages:

1°.—J'ai pu montrer que le mécanisme intime de la Biophotogénèse est le même partout, chez les animaux et chez les végétaux.[1]

2°.—J'ai, en outre, pu choisir dans toute la série des êtres vivants ceux qui présentent le plus d'avantages au point de vue de l'expérimentation: c'est un mollusque lamellibranche, la PHOLADE DACTYLE qui nous a fourni les éléments de recherche les plus importants.

La plus grande difficulté pour les recherches d'ordre Chimique ayant trait à la biophotogénèse est l'infime quantité de substances photogènes contenue dans l'animal ou le végétal lumineux, qui, bien souvent, la consomme au fur et à mesure de sa production, comme c'est le cas des êtres où la lumière est continue (Champignons supérieurs photobactériacées).

Dès 1885, j'avais établi que chez les insectes, la production de la lumière se poursuit pendant un certain temps après que l'on a fait disparaitre toute trace d'organisation cellulaire,[2] en outre j'avais séparé deux substances qui ne brillaient ni l'une de ni l'autre au contact de l'air, quand elles étaient séparées, mais qui émettaient de la lumière quand on les mélangeait.[3] Il n'y avait pas d'oxydation *directe*, bien que la présence de l'oxygène fut nécessaire à l'exercice de la fonction photogène.

Chez l'animal entier (Pyrophorus Noctilucus) ou dans l'organisme lumineux considéré isolément, je reconnus de plus, en 1886, que l'une des deux substances photogènes se comporte comme une Zymase[4] et que, dans son essence même le phénomène ultime, fondamental de toute lumière physiologique est, en dernière analyse, réductible à un processus zymasique.

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[1]Leçons de physiologie générale et comparée, Paris 1898, et traité de physique biologique T. II Paris, Masson 1903. Dictionnaire de physiologie de Richet art. production de LA LUMIERE PAR LES ETRES VIVANTS, Alcan 1912.

[2]V. Elatérides lumineux.

[3]Loc. cit.

[4]Loc. cit.

Plus tard, j'ai pu établir que la Zymase photogène à laquelle ja'i donné le nom de LUCIFERASE, est une péroxydase et qu'elle peut, dans la réaction photogène être remplacée par un peu d'eau oxygénée ou de permanganate de potasse.

La détermination de la nature du second principe photogène, auquel, j'ai donné le nom de LUCIFERINE était particulièrement difficile à établir au moyen des insectes dont les organes lumineux sont de petites glandes à Sécrétion interne (R. Duboix).

Il n'en est plus de même avec la Pholade dactyle qui secrète extérieurement un abondant mucus lumineux et dont le siphon renferme, en outre, en réserve une forte portion de substances photogènes.

On peut résumer de la façon suivante les expériences que j'ai faites autrefois et que j'ai répétées en les complétant et en rectifiant certains points dans ces temps derniers.[1]

(a) Le siphon de la Pholade dactyle avec ses glandes lumineuses est fendu et sèché au soleil. Longtemps après cette opération (plusieurs semaines) on peut rallumer la lumière éteinte dans les glandes en humectant d'eau le siphon dessèché;

(b) Au lieu de déssècher à l'air libre les siphons, on les fend et on les enrobe, encore frais, dans du sucre en poudre fine: ils cessent de briller;

(c) Les siphons confits ainsi conservent pendant plusieurs mois le pouvoir de fournir un liquide très lumineux quand on les fait macérer dans l'eau pendant quelques instants;

(d) le sirop qui résulte de la fonte d'une partie du sucre dans le liquide rejeté par les siphons frais conservés à l'abri de la lumière a donné encore au bout de huit mois un liquide lumineux par son mélange avec trois ou quatre parties d'eau ordinaire;

(e) si l'on introduit dans une théière en grès des fragments de siphons frais ou conservés dans le sucre et que l'on verse dessus de l'eau bouillante, qui par son contact avec le vase et les fragments de siphons, tombe repidement à 70° environ, on obtient un infusum non lumineux;

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[1]Nouvelles recherches sur la lumière physiologique C. R. Ac. des Sc. t. 153 p. 690, Paris 1911.

(f) ce liquide ne brille pas par agitations en présence de l'air: C'est le liquide A;

(g) si, d'autre part, on fait macérer dans de l'eau salée tiède, en agitant de temps en temps des fragments de siphons confits, on obtient un liquide lumineux qui finit par s'éteindre et ne plus briller au contact de l'air par agitation, c'est le liquide B;

(h) si l'on melange les deux liquides A et B la lumière apparait;

(i) L'action photogène du liquide B peut être remplacée par une parcelle de permanganate de potasse ou par un peu d'eau oxygénée neutre;

(j) Si l'on chauffe à 100°, et même à une température peu supérieure à 70° le liquide A, ne donne plus aucune lumière avec le liquide B, ni par le permanganate de potasse ou par l'eau oxygénée: il s'est formé par la chaleur dans liquide A un précipité floconneux;

(k) Il se produit aussi des flocons de coagulation quand on chauffe le liquide B, mais on constate en outre que vers 60°, il perd définitivement tout pouvoir photogène;

(l) La réaction photogène s'opère donc entre deux substances coagulables par la chaleur dont l'une est détruite à 70° et l'autre vers 60°. Si l'on porte à l'ébullition le liquide ou la réaction lumineuse a commencé à se produire et où elle se continuerait à froid pendant longtemps, elle est aussitôt supprimée;

(m) les deux substances photogènes des liquides A et B présentent tous les caractères chimiques et physiques des substances protéiques;

(n) la substance active de A renferme du phosphore et présente les caractères des nucléoprotéines, je lui ai donné le nom de LUCIFERINE;

(o) l'ammoniaque liquide active fortement la réaction photogène. Dans le liquide où s'est opéré la réaction se déposent des cristaux de phosphates. Si à l'ammoniaque on ajoute du sulfate de magnésie, on constate pendant la réaction l'apparition de cristaux de phosphate ammoniaco-magnésien;

(p) Les Siphons frais, sèchés ou confits ne renferment aucune substance LOPOIDE photogène.

(q) La substance active A peut être isolée sans perdre son pouvoir photogène par précipitation à l'aide d'une solution faible d'acide picrique, dont elle doit être séparée immédiatement par filtration. Le précipité recueilli sur le filtre et repris par l'eau brille avec le permanganate de potasse;

(r) toutes les causes physiques ou chimiques qui favorisent, retardent, entravent ou suppriment les réactions zymasiques agissent de même sur le mélange de A et B

(s) Le principe actif de B jouit des propriétés générales des Zymases; il présente en outre les caractères d'une péroxydase, je lui ai donné le nom de LUCIFERASE.

Cette péroxydase n'est pas spéciale aux organismes photogènes, car on peut provoquer la lumière dans le liquide A renfermant la luciférine au moyen du sang de divers animaux à sang froid (Mollusques, Crustacés marins).

(t) Je n'ai pas, au contraire, rencontré de luciférine malgré de nombreuses recherches, en dehors des animaux photogènes.

(u) Le sirop photogène résultant du contact du siphon avec le sucre en poudre est louche; au bout de plusieurs mois de repos dans l'obscurité, on voit monter à sa surface une couche crêmeuse brun jaûnatre. On y trouve en abondance des granulations semblables à celles que l'on rencontre partout dans les organes photogènes; par leur contact avec l'eau, ces granulations prennent la forme de VACUOLIDES découvertes par moi en 1886. Ces éléments actifs *ultimes* de la matière vivante ou bioprotéon ne sont autre chose que ce que l'on a nommé depuis " mitochondries; " le nom de vacuolide que je leur ai donné il y a un quart de siècle est préférable à celui de mitochondrie, en ce sens qu'il indique nettement la nature morphologique de ces *bioultimates* d'une part et leur mode de fonctionnement d'autre part. Ils sont analogues pour toutes les *macrozymases* dont la *purpurase* est le type.[1]

En résumé: *le phénomène fondamental auquel peut être réduit, en dernière analyse toute réaction photogène chez organismes vivants résulte du conflit d'une peroxydase, la " Luciferase " avec*

[1]V. Les vacuolides de la purpurase et la théorie vacuolidaire. C. R. ac. des Sc. T. CLIII p. 1507, 1912.

une matière protéique phosphorée la "Luciferine." Il s'agit donc d'une réaction Zymasique produisant une Oxydation INDIRECTE.

A côté de ces substances, j'en ai rencontré dans les organes photogènes du Pyrophore une autre qui joue aussi un rôle dans le fonctionnement photogénique, mais seulement un rôle de perfectionnement. C'est une substance *fluorescente* à laquelle est dû l'éclat si particulier de la lumière de ces beaux insectes des Antilles. Elle transforme des radiations obscures en radiations éclairantes, ce qui offre plusieurs avantages: 1°.—de diminuer l'énergie perdue en radiations non éclairantes; 2°.—d'éviter l'action nuisible d'une partie des rayons ultra-violets; 3°.—d'accroitre le pouvoir éclairant en lui donnant des qualités spéciales. Je lui ai donné le nom de PYROPHORINE. Je n'ai pu déterminer exactement sa nature et sa composition en raison de la très petite quantité que l'on en trouve dans les Pyrophores, mais il est probable qu'il s'agit d'une glucoside ou peut-être d'un alcaloide. L'acide acétique lui fait perdre sa fluorescence, mais l'ammoniaqué la lui restitue. On peut recommencer plusieurs fois de suite, comme si la pyrophorine formait avec l'acide acétique un sel non fluorescent. J'ai rencontré aussi, plus tard, une substance fluorescente donnant dans l'ultra-violet une belle fluorescence bleue chez un lampyride LUCIOLA ITALICA.[1]

Dernièrement M. M. Ives et W. Coblentz,[2] qui vraisemblablement ignoraient mes travaux ont trouvé également une substance présentant une belle fluorescence bleue. Chez un lampyride américain (Photinus pyralis) et ont pensé à tort que la priorité de la découverte d'un principe fluorescent chez les insectes lumineux leur appartenant.[3]

M. McDermott a signalé aussi la présence d'une matière fluorescente chez divers autres lampyrides américains.[3]

Ce dernier s'inspirant des termes de LUCIFÉRASE et de LUCIFÉRINE dont je me suis servi a proposé pour désigner le principe

[1]Rech. sur la Pourpre et s quelques pigments animaux. Arch. Zool. gén. exp. 5ème Série II, 1909.

[2]Luminous efficiency of the Firefly Bul. Bureau of Standards, t.VI n° 3 1910.

[3]De la fluorescence chez les insectes lumineux C. R. Ac. des Sc. et Sur l'Existence et le rôle de la fluorescence chez les insectes lumineux C.R. de l'A.F.A.S. Dijon 1911.

fluorescent des lampyrides américains la dénomination de LUCIFÉRESCEINE dont la terminaison rappelle celle de la FLUORÉSCEINE. Cette appellation me parait très acceptable et peut être étendue à touses les substances fluorescentes qui peuvent se rencontrer chez les animaux photogènes. On dira: les LUCIFÉRESCEINES, et si l'on voulait désigner particulièrement celle du Pyrophore noctiluque, on pourrait dire la *Pyroluciférescein*e, la *Photinoluciférescein*e, etc.

De ces diverses conditions nait une lumière spéciale dont les propriétés physiques ont été fixées exactement par moi en 1886, et les belles recherches de VERY et LANGLEY en Amérique, n'ont fait que confirmer l'exactitude des recherches que j'ai publiées en 1886 sur la lumière des PYROPHORES des Antilles. Cette admirable LUMIERE FROIDE, réalise sur toutes les autres sources, un énorme avantage puisque son rendement est presque de 100 pour 100 alors que pour nos meilleurs foyers, il n'est guère que de 4 à 5 pour cent.; d'ailleurs; d'une manière générale, l'économie des machines vivantes est bien supérieure à celle des autres.

La LUMIERE FROIDE *est la lumière de l'avenir. Celle que produisent les êtres vivants et supérieure à toutes les autres actuellement usitées et son mécanisme chimique intime est aujourd'hui connu.*

(*Recherches sur les Zymases*)

LES VACUOLIDES DE LA PURPURASE ET LA THEORIE VACUOLIDAIRE

PAR M. RAPHAEL DUBOIS

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Dans un récent et très remarquable travail[1] M. Le professeur Grynfeltt a donné une excellente description de la glande à pourpre dans laquelle on peut lire: " Les sphérules élémentaires et les boules granuleuses ont été surtout bien vues par Raphaël Dubois et répondent sans aucun doute à ce qu'il appelle les vacuolides." Mais le savant anatomiste de Montpellier n'a pas cru devoir conserver ce terme que j'avais employé[2] pour désigner les sphérules élémentaires parce que, dit-il, " dans l'esprit de M. R. Dubois les vacuolides sont les parties élémentaires du bioprotéon," tandis que pour M. Grynfeltt "ces sphérules représenteraient non le bioprotéon lui-même, mais des produits de son activité."[3]

Il m'a semblé que cette divergence de vues appelait une explication de ma part.

J'ai toujours, en effet, soutenu que mes vacuolides étaient les parties élémentaires du bioprotéon, c'est-à-dire les unités morphologiques et physiologiques les plus petites qui soient connues. Mes observations m'ayant conduit depuis longtemps[4] à admettre

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[1]Sur la glande hypobranchiale du Murex trunculus (Bibliographie anatomique, t. XXI, fasc. 4, Berger-Levrault et C°, édit Paris).

[2]Recherches sur la pourpre et sur quelques autres pigments animaux (Arch. de Zool. exp. et gén. 5è série, T. II, n° 7 1909. p. 503 et Fig. I).

[3]" J'ai donné le nom de bioprotéon à ce qu'on appelle communément matière vivante et qui n'est pour moi qu'un état particulier, transitoire, de ce principe unique, essentiellement protéique, à la fois force et matière, énergie et substance, qui, par ses innombrables et incessantes métamorphoses, donne à la nature son infinie variété et que j'ai pour cette raison appelé jadis protéon." (Leçons de physiologie générale et comparée, 1898 p. 7).

[4]Leçons de Physiologie générale et comparée, 1898, p. 74-75, Paris.

que les leucites dérivent des vacuolides[1] et, d'autre part MM. Fauré-Fremiet et Guilliermont admettant qu'ils viennent des mitochondries, il est superflu de se demander si les mitochondries récentes et les vacuolides anciennes ne sont pas une seule et même chose ou, plutôt, deux formes différentes d'un même élément primordial, la granulation collçidale bioprotéonique, dont j'ai décrit et même figuré la structure et le fonctionnement physiologique dans de nombreuses publications depuis 1887[2] car deux quantités égales à une troisième sont égales entre elles.

La plupart on trait à la luciférase, mais la purpurase se comporte de même. Cette dernière, préparée par le procédé qui m'a permis de la découvrir et de l'étudier, contient en abondance les sphérules élémentaires de M. Grynfeltt, c'est-à-dire les vacuolides zymasiques de M. R. Dubois. Ces dernières ne sont pas des produits de fabrication de la cellule, mais bien au contraire, la partie active, agissante du bioprotéon des cellules purpuripares. On voit nettement au microscope que ces vacuolides absorbent les prochromogènes que j'ai appelés *purpurines* et qu'elles les transforment en chromogènes. Le passage du chromogène à l'état de pigment peut se faire dans la vacuolide ou bien en dehors d'elle, par une simple action chimique provoquée par la lumière (Murex brandaris) ou par la chaleur (Murex Trunculus). C'est le mode de fonctionnement des leucites qui est reproduit ici en plus petit.

Les vacuolides de la purpurase, comme toutes les autres naissent, évoluent et se multiplient de la même manière.

Le bioprotéon, ou matière vivante, est du protéon à l'état colloidal, c'est-à-dire composé de particules en suspension. Dans un microbe, il y en a d'innombrables quantités, suffisantes pour expliquer que ces particules ancestrales suffirtont à assurer le

[1]Les vacuolides (C. R. de la Soc. de Biol. T. LX, p. 526) et remarque etc. (Ibid. 1906, p. 528.)

[2]Les vacuolides (C. R. de la Soc. de Biol. 8e série, t. IV 1887) Les élatérides lumineux (Bull. de la soc. Zool. de France, Fig. 7 et 8, pl. IX) anat. & phys. de la Pholade dactyle (Ann. de l'U. de Lyon, 2è Fasc., t. II, Pl. XV, 1892); la lumière physiologique (Revue gen. des Sc. p. et App. 1894, P. 532); recherches sur la pourpre et autres pigments animaux (Arch. Zool. exp. et gen. 5è série, t.II, n°7 1909, p. 503, fig. I).

fonctionnement de toute la lignée. Ces particules ne sont pas toujours visibles à l'ultramicroscope; on les voit apparaître là où il n'y avait rien l'instant avant. Ce sont les particules invisibles qui, en se développant et en se multipliant par divisions, fournissent les granulations visibles, mais dont on ne peut encore distinguer la structure; à leur tour, ces dernières deviennent les vacuolides, dont l'une des espèces les plus grosses est celle de la purpurase; à un degré développement de plus élevé, viennent les leucites. C'est pour ce dernier motif, que, dans la Note de l'Académie dans laquelle j'annonçais la découverte de la purpurase[1] j'ai dit que cette dernière était une *macrozymase.* Cette découverte d'une macrozymase, qui montre nettement que les zymases dont des ferments figurés à une grande importance d'abord au point de vue de la mèrphologie; mais ensuite et sur tout parce que *la macrozymase de la pourpre nous a permis de fournir la première explication du mode d'action des zymases pour laquelle, au lieu de faits d'observation, à la portée de tout le monde, on n'avait, jusqu' à nous, apporté que des hypothèses plus ou moins ingénieuses mais sans fondement contrôlable.*

J'ajouterai que la purpurase, suivant des influences diverses, fixation, dessiccation, coagulation, hydratation, coloration, peut prendre de multiples apparences décrites par les auteurs comme appartement au cytoplasme lui-même.

CONCLUSIONS.—*Les sphérules élémentaires décrites par M. Grynfeltt dans les cellules purpuripares sont identiques aux vacuolides de la purpurase de R. Dubois.*

La purpurase n'est pas un produit de l'activité cellulaire, elle résulte du développement de granulations bioprotéoniques actives par accorissement et multiplication. Elle *a toutes les propriétés des zymases et posédé la structure vacuolidaire. Au point de vue morphologique et physiologique ces vacuolides se comportent comme des leucites, lesquels ne sont que des vacuolides amplifiées.*

La purpurase présente, sous certaines influences, beaucoup d'apparences diverses attribuées au cytoplasme lui-même.

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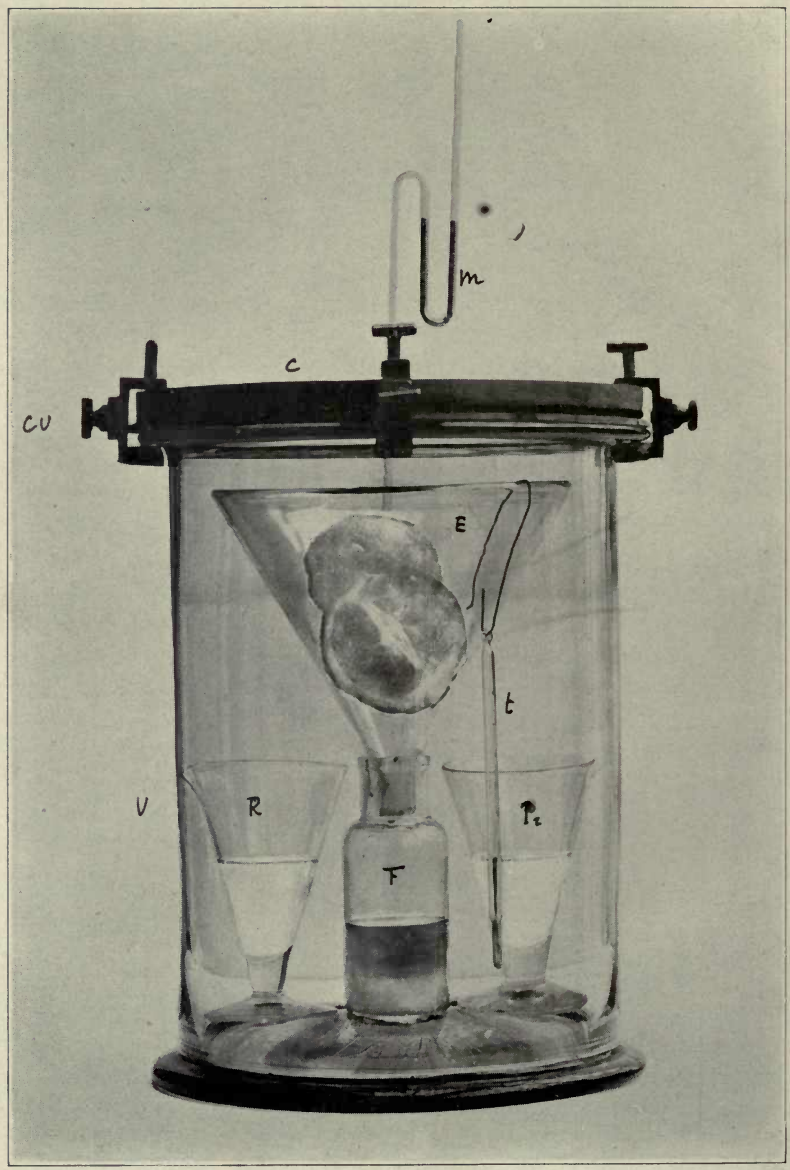
[1]Sur le mécanisme de la formation de la pourpre (Comptes rendus, t.134, 1902, p. 245-247).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PHARMACOLOGIE ET CHIMIE BIOLOGIQUE

ATMOLYSE ET ATMOLYSEUR

Par M. Raphael Dubois

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En raison de l'importance croissante prise en pharmacologie, par les " *intrait* " je crois devoir présenter la note suivante.

J'ai donné le nom d'Atmolyse (atmos. vapeur) à l'action qu'exercent sur la substance organisée les vapeurs des liquides organiques neutres volatils, tels que chloroforme, benzine, éthers, alcools, etc., dont l'inhalation est susceptible de produire l'anesthésie générale.[1]

Les recherches expérimentales, dont j'ai publié les résultats, principalement en 1883 et 1884 dans les Comptes rendus de la Société de Biologie, m'ont conduit non seulement à donner une explication rationnelle expérimentale et très généralement adoptée aujourd'hui du mécanisme intime d'action des anesthésiques généraux; mais encore à montrer les relations étroites, existant entre l'action du froid et celle des anesthésiques,[2] préparant ainsi, par surcroit, l'heureuse application de l'éthérification au forçage des plantes.[3]

Des principes que j'ai découverts sont nés encore d'autres applications qui, dans ces dernières années, ont pris, au point de vue de l'analyse immédiate des tissus organiques et de l'extraction de leurs principes actifs, une grande importance et donné lieu à de nombreuses publications où l'on présente comme des nouveautés ce que j'ai montré il y a un quart de siècle.

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[1]Voir influence des vapeurs anesthésiques sur les tissus vivants (Comptes rendus 1886) et Mécanisme de l'action des anesthésiques (Revue gén. des Sc. p. et app. t. II, 1891, p. 562-565).

[2]Comptes rendus, 26 mai 1912.

[3]Comptes rendus du Congrès de l'A.F.A.S.Lyons 1906.

En outre, on a confondu à tort sous les noms d'éthérolyse, de plasmolyse, d'autolyse, etc., deux procédés absolument distincts, donnant des résultats très différents. L'éthérolyse est le procédé d'extraction des sucs végétaux par immersion dans l'éther liquide, imaginé par Legrip en 1876: ce n'est pas l'atmolyse de R. DUBOIS.

L'expérience suivante montre bien la différence existant entre ces deux méthodes:

On partage deux mandarines en deux et l'on immerge deux des moitiés dans un vase renfermant de l'éther: les deux autres moitiés sont placées dans mon atmolyseur.

Ce dernier se compose d'un vase de verre cylindrique, hermétiquement fermé par un couvercle de verre muni d'un bourrelet de caoutchouc et solidement fixé par des chevalets de cuivre à vis au moyen d'un cadre arrondi de bois dur.

Dans l'intérieur est un entonnoir en verre très évasé, placé sur un flacon destiné à recevoir le liquide atmolysé; de chaque côté sont des récipients destinés à contenir des liquides générateurs de vapeurs atmolysantes, simples ou conjugués. Un manomètre indique la tension des vapeurs et un thermomètre, la température. Les tissus à atmolyser sont suspendus dans l'entonnoir, de façon à éviter le tassement et l'obstruction de la douille.

Dans le flacon d'éther renfermant les moitiés de mandarine, il se forme une couche inférieure aqueuse, colorée, amère et fortement aromatisée par l'essence des cellules de l'épicarpe dissoute par l'éther; c'est le liquide ethérolysé; au contraire, le liquide atmolysé est clair, incolore, sucré et ne contient pas d'essence, celle-ci n'ayant pas été chassée des cellules par les vapeurs d'éther employées comparativement à l'éther liquide.

On pourrait multiplier ces exemples.

Les vapeurs atmolysantes chassent des tissus principalement l'eau[1] qui entraine avec elle surtout des cristalloïdes et parfois même des colloïdes, par exemple, des zymases telles que la luciférase (Dubois), le ferment hépatique (Dastre).

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[1]Voir Fonction d'hydratation (Dictionnaire de Physiologie de Richet); Paris, Alcan, 1909.

Les déplacements d'eau et de principes immédiate primitivement séparés dans la même cellule ou dans des cellules différentes peuvent donner naissance à des produits qui ne préexistaient pas à l'état normal et peuvent exercer une action toxique. C'est ce que j'ai montré en 1883 (loc. cit.) pour les graines de moutarde et les feuilles de lauriercerise atmolysées. Les expériences de Guignard[1] de Mirande,[2] d'Heckel (de Marseille),[3] les observations de Demaussy, de Pougnet, de Molisch, cités par Guérin[4] ne sont que la confirmation de mes expériences anciennes et aussi de l'identité de l'action du gel et de celle des anesthésiques généraux que j'ai, le premier, fait connaître. J'ai montré, en outre, que les vapeurs atmolysantes des anesthésiques généraux se fixent de préférence, et s'accumulent par élection dans les parties riches en lipoïdes (jaune de l'oeuf), semences végétales.[5] Cette importante remarque est, en général, à tort, attribué à M. Overton.

L'atmolyse est un phénomène osmotique se produisant par des échanges entre des liquides aqueux et des vapeurs au travers d'un septum plus ou moins dense. Les vapeurs sont d'autant plus atmolysantes pour l'eau que la chaleur spécifique des liquides qui les fournissent est moins élevée, que leur poids atomique est plus considérable et que toutes les propriétés physiques qui varient dans le même sens sont plus accentuées.

Conclusions—*L'atmolyse que j'ai découverte, est le résultat de l'action osmotique exercée sur la substance organisée des tissus par les vapeurs de liquides anesthésiques. Elle ne doit pas être confondue avec l'éthérolyse, la plasmolyse, l'autolyse, etc. Elle constitue un procédé précieux d'analyse physiologique, physicochimique et aussi d'extraction de principes immédiats utilisables en thérapeutique tels que les intraits, etc.*

[1]Comptes rendus, 12 Juillet 1909.
[2]Comptes rendus, 12 Juillet 1909.
[3]Comptes Novembre 1909 et Juillet 1910.
[4]Rev. Sc. du 24 Déc. 1910.
[5]Comptes rend. de la Soc. de Biol. 19 Mai 1883 p. 376.

UEBER EINIGE CHEMISCHE REAKTIONEN DER MIKROORGANISMEN UND IHRE BEDEUTUNG FÜR CHEMISCHE UND BIOLOGISCHE PROBLEME

VORTRAG VON PROFESSOR DR. FELIX EHRLICH

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Dass Mikroorganismen wie Hefen, Schimmelpilze und Bakterien auf einer grossen Anzahl anorganischer und organisher Stickstoffverbindungen bei Gegenwart der sonst noch erforderlichen Nährsalze normal wachsen und mehr oder minder gut gedeihen können, war den Pflanzenphysiologen und Gärungschemikern seit langer Zeit bekannt. Weniger beachtet wurden aber noch vor kurzem die für den Lebensprozess so wichtigen chemischen Vorgänge bei der Stickstoffassimilation deiser kleinen Lebewesen, vor allem die weitgehenden Veränderungen, die während des Wachstums das Nährsubstrat selbst und die darin ursprünlich enthaltenen Stickstoffsubstanzen erleiden.

In Jahre 1905 habe ich zuerst bei Gelegenheit der Aufklärung der Fuselölbildung[1] auf die eigentümlichen tiefgreifenden chemischen Umwandlungen von Aminosäuren durch gärende und assimilierende Hefe nachdrücklich hingewiesen. Aus meinen Untersuchungen ging zunächst hervor, dass die Aminosäuren, die entweder direkt in der Lösung vorhanden sind oder sich durch enzymatische Prozesse aus dem Eiweiss des Nährmediums oder der Hefe selbst abgespalten haben, unter natürlichen Bedingungen und auch unter den Bedingungen der technischen Gärung die eigentlichen wichtigsten Stickstoffnährstoffe der hefe darstellen, was bis dahin keineswegs mit hinreichender Genauigkeit klargestellt war. Der exakte Beweis, dass die natürlich vorkommenden Aminosäuren wirklich von gärender Hefe aus der Lösung aufgenommen und auf Eiweiss verarbeitet werden, gelang

[1] F. Ehrlich, Zeitschrift des Vereins der Deutschen Zuckerindustrie **55**, 539-567 (1905).

mit Hilfe der von mir ausgearbeiteten Gärmethode zur asymetrischen Spaltung der Racemverbindung der betreffenden Aminosäuren durch Hefe,[1] bei deren Anwendung in fast allen Fällen eine Aktivierung der vorgelegten Stickstoffsubstanz die tatsächlich erfolgte Assimilation der optisch aktiven in der Natur auftretenden Komponente durch den Hefepilz mit Sicherheit anzeigte.

Von besonderem Interesse für die Kenntnis Physiologie der Mikroorganismen war nun das durch die weiteren Untersuchungen gezeitigte Resultat, dass Hefe bei der Eiweissbildung die Aminosäuren des Nährsubstrates, auf dem sie wächst, ihrem Körperprotein nicht direkt durch Kondensation etwa nach Art der Polypeptidsynthesen Emil Fischers einverleibt, wie man bis dahin anzunehmen geneigt war. Vielmehr liess sich regelmässig beobachten, dass die gärende Hefe das Molekül der Aminosäuren bei der Assimilation spaltet, den dabei freiwerdenden Stickstoff in Form von Ammoniak für ihren Eiweissaufbau verwertet, den grössten Teil des stickstoffreien Moleküls aber in Form von Alkoholen unverwertet in der vergorenen Lösung zurücklässt. Es entsteht auf diese Weise, wie sich zeigte[2], aus Leucin inaktiver Iso-Amylakohol, aus Isoleucin optisch aktiver d-Amylalkohol, aus Valin Isobutylalkohol d.h. die Hauptbestandteile der Fuselöle der Hefegärung bilden sich aus den in grosser Menge in jedem Eiweiss vorkommenden Aminosäuren. Auf Grund dieser Befunde liess sich dann direkt eine Gärungsgleichung für die Fuselölbildung entwickeln, die folgendem Schema entspricht:

$$R.CHNH_2CO_2H + H_2O = R.\ CH_2OH + CO_2 + NH_3$$

Auf ähnliche Weise war auch die Entstehung der Bernsteinsäure bei der alkoholishcen Gärung herzuleiten nur mit dem Unterscheide, dass hier der intermediär aus der Glutaminsäure als Muttersubstanz hervorgegangene Alkahol eine weitergehende Oxydation zur entsprechenden Dikarbonsäure erfährt.[3]

Die für die Fuselölbildung aufgestellte Gleichung wurde nun direkt der Pfadfinder für die Entdeckung einer ganzen Anzahl

[1]F. Ehrlich, Biochemische Zeitschr. **1**, 8-31 (1906); **8**, 438-466 (1908).

[2]F. Ehrlich, Berichte der Deutschen Chemischen Gesellschaft **40**, 1027-1047; **40**, 2538-2562 (1907).

[3]F. Ehrlich, Biochemische Zeitschr. **18**, 391-423 (1909).

von Gärungsalkoholen denn alle α-Aminosäurenunterliegeneinem analogen Abbau durch gärende Hefe genau wie die Ursubstanzen des Fuselöls. Man kann also direkt von einer "alkoholischen Gärung der Aminosäuren" sprechen, die normaler Weise stets parallel neben der alkoholischen Gärung des Zuckers verläuft in dem Masse, wie die wachsende und gärende Hefe den Aminosäuren des Nährmediums den Stickstoff zum Aufbau ihres Körpereiweisses entzieht. Unter den neu aufgefundenen Alkoholen, die man durch Hefegärung sehr leicht und bequem präparativ darstellen kann und die je nach Herkunft mit dem Stammwort der betreffenden Aminosäure benannt wurden, sind mehrere von besonderem Interesse. Wie das Tyrosol (p-Oxyphenylaethylalkohol),[1] das Tryptophol (-Indolylaethylalkohol)[2], das Histidol und andere, deren Untersuchung noch nicht abgeschlossen ist. Offenbar haben viele von diesen Alkoholen als solche oder in Form bestimmter Ester einen hervorragenden Anteil an dem Zustandekommen des Geschmacks und Aromas der gegorenen Getränke, besonders des Bieres und Weines.

Dass die alkoholische Gärung der Aminosäuren auf enzmatische Prozesse ähnlich wie die Zuckergärung zurückführen ist, lässt sich mit grosser Wahrscheinlichkeit annehmen. Die Gesamtreaktion setzt sich scheinbar aus einer Summe von einzelnen Enzymwirkungen zusammen wie Hydratationen, Ammoniak-und Kohlendioxyd-Abspaltungen, etc., die jede für sich wohl gelegentlich beobachtet, die aber in ihrer Gesamtheit bis dahin nicht bekannt waren. Der Nachweis derartiger Enzyme gelang allerdings bisher nicht. Weder abgetötete Hefe noch Hefepressaft vermögen Aminosäuren in Alkohole zu verwandeln.[3] Est ist also anzunehmen, dass es sich hier um sehr empfindliche im Stoffwechsel der Hefe tätige Enzyme handelt, für deren Abtrennung vom Leben der Hefe unsere jetzige Methodik der Fermentforschung noch nicht ausreicht. Veilleicht ist die von C. Neuberg[4] neuerdings entdekte Carborylase, die Ketosäuren wie die Brenztraubensäure in Aldehyde und Kohlendioxyd spaltet

$$R.\,CO.\,COOH = CO_2 + R.\,CHO,$$

[1]F. Ehrlich, Ber. d. Deutsch. Chem. Ges. **44**, 139-146 (1911).
[2]F. Ehrlich, Ber. Deutsch. Chem. Ges. **45**, 883-889 (1912).
[3]F. Ehrlich, Ber. Deutsch. Chem. Ges. **39**, 4072-75 (1906).
[4]Biochem Zeitschr. **36**, 76 (1911).

eines der vielen bei der alkoholischen Gärung der Aminosäuren wirksamen Enzyme. Die Möglichkeit eines solchen Reaktionsverlaufes, die allerdings erst noch genauer zu beweisen wäre, ist nicht unwahrscheinlich, nachdem O. Neubauer und Fromherz[1] die glatte Vergärbarkeit von Ketosäuren durch lebende Hefe zu denselben Alkoholen wie aus den entsprechenden Aminosäuren gezeigt haben

$$R . CHNH_2 . CO_2H \rightarrow R . COCOOH \rightarrow R . CH_2OH$$

und auf Grund dieser Untersuchungen die Ketosäuren, deren Isolierung auch in einen Falle gelang, als Zwischenprodukte der Bildung von Fuselöl und andern Alkoholen aus Aminosäuren auffassen. Die Ueberführung von Aldehyden in die entsprechenden Alkohole erscheint jedenfalls als enzymatischer Teilvorgang bei der alkoholischen Hefegärung nicht mehr befremdlich, nachdem C. I. Lintner und v. Liebig[2] die Hydrierung von Furfurol zu Furfuralkohol durch gärende Hefe durchführen konnten.

Als besonders bemerkenswert ergab sich dann noch bei meinen weiteren Untersuchungen, dass der Abbau von Aminosäuren zu Alkoholen durch gärende Hefe nur bei Gegenwart von Zucker vor sich gehen kann. Ohne Zucker zu vergären, ist die Hefe nicht imstande, Aminosäuren zu assimilieren oder überhaupt nur anzugreifen, und es hat sich bisher für Kulturhefen keine Substanz ergeben, welche die gärfähigen Kohlehydrate in dieser Hinsicht vollwertig ersetzen kann. Der Zucker bildet offenbar die Energiequelle, mit deren Hilfe die Hefe den Eiweissaufbau vollzeiht, und gleichzeitig das Baumaterial, aus dessen Bruchstücken zusammen mit dem aus Aminosäuren abgespaltenen Ammoniakmolekül bei der Gärung die Synthese des Körperproteins der Hefe erfolgt. Die alkoholische Gärung des Zuckers hat also scheinbar nur die Bedeutung eines allerdings sehr wichtigen Faktors bei der Plasmabildung der Kulturhefen, während die alkoholische Gärung der Aminosäure als eine notwendige Folge dieser Plasmabildung anzusehen ist, hervorgerufen durch das Unvermögen der Kulturhefen, die nach Abspaltung des Ammoniaks beim Eiweissaufbau des Organismus verbleibenden stick-

[1]Zeitschr. f. physiol. Chem. **70**, 326 (1911).
[2]Zeitschr. f. physiol. Chem. **72**, 449 (1911).

stoffreien Reste der Aminosäuren weiter für den Stoffwechselprozess aufzunnutzen.[1]

Durch ausgedehnte Versuche in grossem Masstabe, die ich mit K. A. Jacobsen ausführte, liess sich dann feststellen,[2] dass nicht allein untergärige, obergärige und Wein-Kulturhefen, sondern auch die verscheidensten wilden Heferassen z.B. des Anomalus-, Torula-, Pichlia-Typhus, Kahmhefen, etc., der gleichen Reaktion der Umwandlung von Aminosäuren in Alkohole fähig sind. Wesentlich anders verlief aber die Einwirkung von Schimmelpilzen and ihnen nahestehenden Organismen auf Aminosäuren. Hier wurden Gruppen von Mikroorganismen gefunden, wie Oidium lactis, Mucoraceen, Monilia-Pilze, etc., welche unter sonst gleichen Bedingunegn wie bei der Hefegärung Aminosäuren nicht zu Alkoholen, sondern im wesentlichen zu *Oxysäuren* verarbeiten. ent sprechend der allgemeinen Gleichung:

$$R . CHNH_2 . CO_2H + H_2O = R . CHOH . CO_2H + NH_3$$

Diese Umwandlung verläuft bei einzelnen Schimmelöuilzen wie Oidium lactis und für einzelne Aminosäuren mit aromatischem Kern derartig quantitativ, dass sie zur präparativen Darstellung der betreffenden Oxysäuren, z.B. p-Oxyphenylmilchsäure, Phenylmilchsäure, Indolmilchsäure dienen kann, die auch bei Anwendung von racemischem Ausgangsmaterial stets optisch aktiv erhalten werden. Einzelne Arten wie Monilia candida vermögen aus Aminosäuren sowohl Alkohole wie Oxysäuren zu bilden, andere dagegen wie Penicillium glaucum, Aspergillus niger und manche Mucor-Rassen bauen die ursprünglich entstehenden Oxysäuren weiter zu niedrigermolekularen Verbindungen ab. Von diesem tiefgehenden Abbau werden namentlich die Aminosäuren der Fettreihe wie Leucin, Glutaminsäure, etc., betroffen, während die Aminosäuren mit aromatischer Gruppe wie Tyrosin, Tryptophan, etc., dem Angriff einzelner Schimmelpilze viel länger Widerstand leisten. Auch hier finden sich allerdings einige Penicillium-, Aspergillus-, Arten und bestimmte

[1]F. Ehrlich, Landw. Jahrbücher 1909, V, 289-327, sowie F. Ehrlich, " Ueber die Bedeutung des Eiweisstoffwechsels für die Lebensvorgänge in der Pflanzenwelt " in Herz's Sammlung chem. u. chem.-techn. Vorträge Stuttgart 1911 Bd. XVII.

[2]F. Ehrlich-u. K. A. Jacobsen, Ber. Deutsch. Chem. Ges. **44**, 888-897 (1911).

Bakterien, die Tyrosin bis zum Verschwinden der Millon'schen Reaktion zersetzen d.h. also eine vollständige Aufsprengung des Benzol-Ringes herbeiführen.

Als eine gemeinsame sehr bemerkenswerte Eigentümlichkeit der Schimmelpilze sowohl wie der wilden hautbildenden Hefen stellte es sich nun heraus, dass diese Gruppen von Mikroorganismen ganz im Gegensatz zu den Kulturhefen Aminosäuren selbst dann angreifen und zu Oxysäuren und Alkoholen abbauen, wenn andere Substanzen wie Zucker zugegen sind.[1] Ausser Kohlehydraten können nämlich die betreffenden Organismen auch Verbindungen wie Glycerin, Milchsäure, Aethylalkohol, aber auch viele andere Substanzen der Fettreihe als Kohlenstoff- und Energiematerial für ihren Eiweissaufbau verwerten, wobei aus den Aminosäuren die gleichen Abbauprodukte wie bei Gegenwart von Zucker resultieren. So bildet aus Tyrosin die Heferasse Willia anomala Hansen Tyrosol und Oidium lactis aus derselben Aminosäure p-Oxyphenylmilchsäure in fast denselben Mengenverhältnissen, gleichgültig, ob neben Tyrosin Zucker, Glycerin oder Aethylalkohol als Kohlenstoffmaterial geboten ist. Die Willia-Hefe produziert sogar deutlich, wenn auch in geringen Quantitäten, Tyrosol, wenn nur Methylalkohol oder Amylalkohol neben Tyrosin in der Lösung vorhanden ist. In allen diesen Fällen war auch stets ein deutliches Wachstum des betreffenden Organismus wahrnehmbar. Diese Merkwürdige Erscheinung erklärt, warum bei vielen wilden Heferassen und Schimmelpilzen unter Umständen ein tiefergehender Abbau der zunächst aus den Aminosäuren gebildeten Säuren oder Alkoholen erfolgen kann, da namlich diese primären Abbauprodukte unter entsprechend ungünstigen Bedingungen leicht weitere Verwertung bei der Eiweissynthese der Organismen erfahren. Die Beobachtung, dass gewöhnlicher Alkohol bei der Ernährung hautbildender Hefen und Schimmelpilze einen vollwertigen Ersatz für Zucker bilden kann, gibt überdies eine bequeme Methode an die Hand, besonders empfindliche oder sehr leicht lösliche Stoffwechselprodukte aus Aminosäuren besser und in reinerer Form zu isolieren, da bei Verwendung von Alkohol viele häufig die Isolierung

[1] F. Ehrlich, Biochem. Zeitschr. **36**, 477-497 (1911).

störende Nebenprodukte aus Zucker bei der Aufarbeitung der Nährlösungen wegfallen.

Die eigenartigen Umwandlungen, die Aminosäuren durch Hefen und Schimmelpilze erleiden, ermutigten nun weiterhin dazu, die Einwirkung dieser Mikroorganismen auf andere Organische stickstoffhaltige Substanzen zu studieren. In dieser Hinsicht erschienen zunächst die *primären Amine* von Interesse, unter denen bekanntlich einige bei der Fäulnis von Aminosäuren auftreten. Untersuchungen, die ich gemeinsam mit P. Pistschimuka in dieser Richtung unternahmé,[1] dass diese Verbindungen von wilden Hefen und von vielen Schimmelpilzen fast quantitativ, weniger leicht dagegen von Kulturhefen analog den Aminosäuren in *Alkohole* übergeführt werden, entsprechend der Gleichung:

$$R . CH_2NH_2 + H_2O = R . CH_2OH + NH_3,$$

wobei, von den Kulturhefen abgesehen, statt Zucker ebenfalls Glycerin, Aethylalkohol, etc., als Kohlenstoffnährmaterial dienen können. So wurde Amylamin in Amylalkohol, p-Oxyphenylaethylamin in Tyrosol umgewandelt, und es erscheint besonders interessant, dass letzteres Amin, das nach Bargers Untersuchungen das giftige Prinzip des Mutterkorns bildet und für den menschlichen und tierischen Organismus so gefährliche Eigenschaften besitzt, von Hefen und Schimmelpilzen ohne jede Schädigung ertragen und sogar für den normalen Stoffwechselprozess äusserst günstig verwertet wird.

Die leichte Ueberfürhbarkeit der Amine in Alkohole durch viele Heferassen legt den Gedanken nahe, ob nicht etwa auch die Fuselölbildung der Hefe aus Aminosäuren einen ähnlichen Reaktionsverlauf nehmen kann, bei dem die Amine die Zwischenprodukte bilden würden zufolge des Schemas

$$R . CHNH_2 . CO_2H \rightarrow R . CH_2NH_2 \rightarrow R . CH_2OH.$$

Da bei der Fäulnis ein Abbau von Aminosäuren zu Aminen unter CO_2-Abspaltung häufig beobachtet ist, so ist die Möglichkeit eines solchen Abbaus auch bei der alkoholischen Gärung der Aminosäure nicht ohne weiteres von der der Hand zu weisen. Wenn Kulturhefen nur in geringem Masse befähigt sind, aus

[1] F. Ehrlich u. P. Pistschimuka, Ber. Deutsch. Chem. Ges. **45**, 1006-1012 (1912).

Aminen Alkohole zu bilden, so spricht diese Erscheinung nicht unbedingt gegen das oben skizzierte Abbauschema, da es wohl denkbar erscheint, dass für Kulturhefen die quantitative Durchführung der Reaktion bis zum Alkohol wesentlich von der gleichzeitigen CO_2-Abspaltung abhängt, der vielleicht bei diesen Hefen die Rolle einer besonderen Energiequelle zukommt.

Neuerdings habe ich dann noch gemeinsam mit Herrn Fritz Lange das Verhalten von Hefe und Schimmelpilzen gegen *tertiäre Amine* einer eingehenden Bearbeitung unterzogen. Es erschien hier besonders von Interesse, ob solche Stickstoffverbindungen im Stoffwechsel von Mikroorganismen ausgenutzt werden können und welche Abbauprodukte dabei gebildet werden. Sehr geeigneerschienen für diese Versuche das Hordenin (p-Oxyphenylaethylt dimethylamin) $p\text{-}OH.C_6H_4.CH_2CH_2N(CH_3)_2$ und das Betain (Trimethylamidoessigsäure) $(CH_3)_3N.CH_2.COO$

Das von Léger in den Malzkeimen aufgefundene Hordenin ist interessant wegen seiner nahen chemischen Beziehungen zum Tyrosin, zum p-Oxtphenylaethylamin und auch zum Tyrosol. Das Betain bildet ein wichtiges Abfallprodukt der Zuckerindustrie in der Melasse und Melasseschlempe. Es wird in den letzten Jahren daraus in grossen Mengen technisch nach einem von mir angegenen Verfahren[1] hergestellt und in Form seines Chlorids, das in wässriger Lösung stark hydrolysiert ist, unter dem Namen Acidol oder mit Pepsin trocken gemischt als Acidol-Pepsin in der Pharmazie als Ersatz für flüssige Salzsäure heute viel benutzt. Das Betain als solches ist gegen chemische Angriffe sehr widerstandsfähig, selbst gegen Königs-Wasser. Nach den Untersuchungen vieler Physiologen wird es vom tierischen und menschlichen Organismus so gut wie garnicht ausgenutzt, und erscheint zum grössten Teil in Harn wieder. Auch über die Verwertung des Betains durch Mikroorganismen liegen bisher nur ganz vereinzelte Angaben vor, nach denen nur sicher gestellt erscheint, dass Brennerei-und Brauereihefen auf Betain nicht gedeihen können.[2]

Durch unsere Versuche haben wir nun zunächst ermittelt, dass sowohl Hordenin wie Betain vorzügliche Stickstoffnährmit-

[1]Deutsches Reichspatent No. 157173.

[2]Stanek, Zeitschr. f. d. ges. Brauwesen **30**, 566 (1907).

tel für eine ganze Reihe von Mikroorganismen bilden und zwar im wesentlichen für dieselben, die auch primäre Amine angreifen, wobei-, Alkohol ebenso wie Zucker als Kohlenstoffmaterial dienen kann. Hierunter gehören vor allem hautbildende und Kahmhefen, wie Willia anomala, Pichia farinosa und membranefaciens, Monilia candida sowie eine ganze Anzahl von Schimmelpilzen wie Oidium lactis und lupuli, Penicillium-und Aspergillus-Arten, Epicoccum purpurascens, Citromyces Pfefferianus, etc. Näher verfolgt wurde der Abbau, den die tertiären Amine durch das Wachstum der Heferasse Willia anomala erleiden. Hierbei zeigte sich merkwürdiger Weise, dass auch in diesen Fällen eine Ersetzung der Amingruppe durch die Hydroxylgruppe stattfindet, und dass bei der Assimilation von *Hordenin* fast quantitativ *Tyrosol*, bei derjenigen von *Betain* deutlich nachweisbar *Glykolsäure* auftritt zufolge der Gleichungen

$$OH \cdot C_6H_4.CH_2CH_2\ (CH_3)_2 + H_2O = OH.C_6H_4.CH_2.CH_2OH + NH(CH_3)_2$$

$$(CH_3)_3N.CH_2COO + H_2O = CH_2OH.COOH + N(CH_3)_3$$

In den Hordenin-und Betain-Lösungen, auf denen die Hefe gewachsen war, liess sich nun aber weder Dimethylamin, noch Trimethylamin nachweisen. Diese Amine scheinen durch Wasseranlagerung einen weiteren Abbau erfahren zu haben unter Bildung von Ammoniak und Methylalkohol etwa im Sinne der Gleichung

$$N(CH_3)_3 + 3H_2O = NH_3 + 3CH_3OH.$$

Auch das hierbei entstehende Ammoniak war in den Lösungen nicht auffindbar. Ganz analog wie bei der Assimilation der Aminosäuren dient offenbar das intermediär abgespaltene Ammoniak für die Eiweissynthese der Pilze, wobei wahrscheinlich gleichzeitig der nebenher gebildete Methylalkohol durch weitere Oxydation ebenfalls Verwertung findet. Hierfür liessen sich triftige Beweise aus dem Verhalten von Willia anomala gegen Trimethylamin und Ammoniak herleiten. Diese Heferasse wächst nämlich auf den anorganischen Salzen dieser beiden Basen sehr üppig, wenn ihr gleichzeitig Zucker oder Aethylalkohol geboten ist, zeigt aber auch deutliche, wenn auch nicht so starke Vegetation, sobald nur Methylalkohol als einzige C-Quelle zugegen ist. Der direkte Nachweis von Ammoniak als Zwischen-

produkt des mikro-biochemischen Abbaus tertiärer Amine gelang dann noch beim Penicillium glaucum, das schon nach kurzem Wachstum auf Betain oder Trimethylamin-Lösungen wahrnehmbare Mengen Ammoniak produziert.

Bei meinem mit F. Lange unternommenen Versuchen bin ich dann noch einen wesentlichen Schritt weitergegangen. Wenn tertiäre und auch quaternäre Aminverbindungen wie das Betain als N-Quelle für Mikroorganismen Bedeutung haben, so war zu erwarten, dass auch *ringförmige Stickstoffverbindungen* in dieser Hinsicht irgendwie in Betracht kommen mussten. In der Tat zeigte sich, dass Pilze wie Willia anomala, Oidium lactis, Pichia farinosa, Penicillium glaucum in mehr oder minder ausgeprägter Weise deutliches Wachstum auf Lösungen von Pyridinphosphat, Piperidintartrat, Cinchoninsäure, etc., zeigen. Besonders überraschend war, dass dieselben Hefen und Pilze auch teils stärker, teils schwächer normale Zellbildung auf einer ganzen Reihe von *Alkaloiden* erkennen lassen z.B. auf Coniin, Chinin, Cocain, Brucin, Nicotin. Wenn es auch hier bisher noch nicht gelang, bestimmte Stoffwechselprodukte bei Verarbeitung der Alkaloide durch die Pilze abzuscheiden, so steht doch zu erwarten, dass unter Einhaltung gewisser Konzentrationen der Nährlösungen bei manchen verhältnismässig üppig wachsenden Schimmelpilzen wie den Penicilliumarten einer solchen Isolierung wohl möglich sien wird. Vielleicht ist hiermit den organischen Chemikern ein neues Hilfsmittel an die Hand gegeben, die Konstitution mancher chemisch noch unbekannter Alkaloide oder ihrer Abbauprodukte näher dadurch zu erforschen, dass man auf den Lösungen der Alkaloide bestimmte Mikroorganismen zur Vegetation bringt und aus der Art des Wachstums und der danach isolierten Spaltungsprodukte Schlüsse auf die Bindungsform des Stickstoffs und die chemische Struktur des betreffenden Alkaloids zieht. Wenigstens haben schon eine Reihe von Vorsersuchen ergeben, dass je nach den Bindungsverhältenissen des Stickstoffs der Angriff von Alkaloiden durch Mikroorganismen sehr verscheiden erfolgt, sodass z.B. Nicotin, das einen leicht aufspaltbaren Pyrrolidin-Ring enthält, eine wesentlich günstigere Stickstoffnahrung für die Pilze bildet als Alkaloide mit fester gefügter Stickstoffgruppe wie Chinin, Cocain, etc.

Wenn auch zur Aufklärung dieser eigenartigen Beziehungen noch sehr eingehende Arbeiten erforderlich sein werden, so ermutigen doch schon die hier mitgeteilten Resultate die verschiedenen chemischenen Reaktionen, deren die Mikroorganismen fähig sind, mehr als bisher zur Erforschung organisch-chemischer Probleme heranzuziehen. In dieser Hinsicht dürfte namentlich die Eindeutigkeit interessant erscheinen, mit der in ganz heterogen zusammengesetzten organischen Stickstoffverbindungen wie primären, tertären Aminen, Aminosäuren, etc., durch sehr viele Arten von Mikroorganismen regelmässig und in zahlreichen Fällen fast quantitativ ein Ersatz des Stickstoffs durch die Hydroxylgruppe erfolgt. Zweifellos werden sich diese Reaktionen in verschiedenster Richtung noch variieren lassen und ähnlich wie jetzt zur präparativen Darstellung von manchen sonst schwer zugänglichen Alkoholen mittels Hefe und Oxysäuren mittels Oidium lactis wird man die biochemische Wirkung der Mikroorganismen noch für die Gewinnung vieler anderer organischer Substanzen vorteilhaft ausnutzen können. Das Arbeiten mit Mikroorganismen sollte daher mehr als es bis jetzt geschehen ist, zum Rüstzeug der Experimentierkunst jedes organischen Chemikers gehören. Die Einrichtungen dafür sind ja in jedem chemischen Laboratorium vorhanden oder leicht zu beschaffen, die Bereitung der Nährlösungen und die Reinzucht der Mikroorganismen sind einfache leicht erlernbare Operationen. Ueber-dies kann man hierbei vorteilhaft mit sehr geringen Mengen Substanzen experimentieren und die relativ niedrigen Temperaturen, bei denen die eigentliche Einwirkung der Mikroorganismen erfolgt, verbürgen ausserdem eine möglichst weitgehende Schonung der zu verarbeitenden Substanz und der daraus erhaltenen Produkte.

Dass die Auffindung von chemischen Reaktionen der Mikroorganismen in der Art der hier geschilderten für viele biologische Probleme von Bedeutung zu werden verspricht, geht ja schon aus den obigen Auseinandersetzungen zur Genüge hervor und bedarf daher nicht einer besonderen Hervorhebung. Man wird jetzt allmählich daran gehen können, für die Mikroorganismen mehr wie bisher einer Systematik auf chemisch-physiologischer Grundlage zu schaffen und dabei als Ausgangspunkte für das

verschiedene biologische Verhalten nicht allein die Kohlehydrate nehmen, wie bis jetzt bei den Hefen, sonedern vor allem die für die Plasmabildung so wichtigen Eiweisstoffe, ihre Spaltprodukte, die Aminosäuren, und die daraus entstehenden je nach der Gattung des Organismes verschieden gebauten Stoffwechsel-Endprodukte. Weiterhin wird dann die verschiedene spezifische Einwirkung auf andere chemische Substanzen einen Massstab für die Einteilung der verschiedenartigen Rassen und Gruppen von Hefen und Schimmelpilzen bilden können. In dieser Hinsicht sei daran erinnert, wie eigenartig und scharf die Gruppe der Kulturhefen sich von den übrigen wilden Heferassen dadurch abhebt, dass sie im Gegensatz zu diesen Hefen Amine und ähnlich konstituierte Verbindungen sogut wie garnicht für ihren Stoffwechselprozess ausnutzen können, sondern nur imstande sind, Kohlehydrate als Kohlenstoffbausteine für die Eiweisssynthese zu verwenden, während Kahmhefen alle möglichen anderen Verbindungen zu diesem Zwecke heranziehen können.

Schliesslich wird nicht zu bezweifeln sein, dass das weitere Studium der Einwirkung von Mikroorganismen in der hier skizzierten Richtung von grosser Bedeutung für die Aufklärung der Stoffwechselprozesse nicht allein in den niederen, sondern auch in den höheren Pflanzen werden kann. Hier sind es namentlich die Fragen der Entstehung der Riechstoffe und Alkaloide und ihres Schicksals in den grünen Pflanzen, zu deren Klärung Vorarbeiten auf dem Gebiete der Biochemie der Hefen und Schimmelpilze nach dem oben entworfenen Arbeitsplane sicher sehr wesentliche Beiträge liefern werden.

(*Abstract*)

THE CHEMICAL CHANGES TAKING PLACE IN MILK UNDER PATHOLOGICAL CONDITIONS

By Lewis W. Fetzer

Department Agriculture, Washington, D. C.

In 1907 a study was begun in the laboratory of physiological chemistry at the Maryland Agricultural Experiment Station of the chemical changes taking place in the milk of animals suffering from inflammations, other than tuberculosis, of the mammary glands. The work was done in conjunction with the veterinary department and was completed in the fall of 1909. Owing to pressure from other duties and employment elsewhere I have not been able to make a complete report upon this topic until the present time.

As we all know, there are numerous analyses of milks of abnormal composition on record, some of which are stated to be of pathological origin, and others which undoubtedly originate from animals having a mammary gland affection. In practically all instances, however, the analytical data which are presented are incomplete and almost always are confined to the constituents which are reported in commercial analysis, i.e., fat, total solids, solids-not-fat, and possibly lactose. Another feature which has not been taken into account when reporting previously recorded analyses is the stage of the disease at which the sample was obtained. This is a very important point which must be considered at all times, if the results obtained are to be utilized.

We were very fortunate in locating a barn which contained a herd in which mastitis existed. The disease had previously appeared from time to time in this barn without leaving any apparent severe effects upon the animals.

The plan followed in this work was the following one: The overseer of the dairy barn and his helpers were instructed by Dr. S. S. Buckley, the veterinarian of the Experiment Station, to report

to him any abnormality which they might note in the behavior of the animals, and furthermore, any appearance of redness, tenderness and hardness on and in the mammary glands. They were, in addition, instructed to report any change in the color and consistency of the milk and the amount of milk obtained.

Our main purpose in setting up a plan of this character was to enable us to study the milk from these animals throughout the cycle of the disease, i.e., from its inception until the time when the mammary gland appeared clinically normal and the milk had assumed its right appearance. In this connection I will say, however, that a milk that had the appearance of being normal and comes from an udder which has apparently healed is no guarantee that the milk is fit for human consumption.

On receiving the report of the dairyman or milker that such and such a cow showed some unusual peculiarity in regard to its gland or the milk therefrom, the milk was drawn in sterile Erlenmeyer flasks or sterile quart milk bottles. If the sample was taken early in the morning it was placed in a refrigerator and taken in work on the same morning as soon as the laboratory opened. If sampled in the late afternoon the milks were placed in the refrigerator and kept until the following morning, when the analysis was begun. Most of the samples of milk were obtained in the morning. In no case was a milk examined which had taken the form of a jelly or contained a compact jelly-like mass. The reason for this was that we wanted to examine the milks which were most liable to be passed on into the milk supply. The observations made were as follows:

General history of animals:

Breed of animals.
Age.
Number of calves.
Frequency of abortion.
Kind of barn kept in previously.
Whether a good milker or not.

Clinical history of animals:

Date of inception of mastitis; physical condition of mammary glands at the beginning and during the cycle of the disease;

whether one or more quarters were affected; the duration of the disease; and if the quarters affected were finally rendered worthless as far as milk production is concerned.

The Milk:

(a) Odor; consistency; whether it contained flakes or stringy pus in suspension blood, etc., or a sediment.

(b) Microscopical examination—the nature of the sediment obtained with the centrifuge.

This part of the examination was very limited, as we had only a very small laboratory force at our disposal. It consisted of obtaining a differential leucocyte count and observations as to the morphological characteristics of bacteria present.

(c) Chemical examination.

In the chemical work the observations and determinations made were as follows: Reaction, total solids, total nitrogen, total protein (nitrogen X 6.37), casein, albumin, globulin, amids, peptones, ammonia, lactose, fat, cholesterol, lecithin, ash, ash constituents; potassium, sodium calcium, magnesium phosphoric acid, chlorin, sulphuric acid and iron.

Enzym tests: Catalse, peroxidase, oxidase and reductase.

The principal changes noted in the chemical composition of the milk during the pathological process were as follows:

Acidity: Most milks at the beginning of the process showed a diminution of the apparent acidity. This in some instances went on until alkalinity set in, where it remained until the disease began to undergo resolution, after this the acidity gradually rose to its normal point again.

Total solids: In acute cases the total solids were high at the outset, but as the process went on there was a diminution of the same.

Total solids-not-fat: In some instances there was an increase, while in others no marked change took place.

Total nitrogen and protein X 6.38. Increased at the outset and remained increased until resolution took place.

Casein: Diminished in some instances and remained so until the pathological condition was eliminated.

Lactglobulin? (*Serum globulin*): Increased until resolution set in.

Albumin? (*Serum albumin*): Increased during the whole process, then back to normal.

Lactose: Diminished gradually as the process went on, then back to normal.

Fat: Diminished gradually until the fastigium of the process was reached, then increased again.

Cholesterol: Diminished gradually until the fastigium of the process was reached, and then increased again. When calculated on the basis of 100 parts of ether extract it seemed to be increased.

Lecithin: Diminished gradually, then increased gradually as resolution was taking place.

Ash: In some cases an increase took place, but only in a few instances was a very large increase apparent.

Ash constituents: The most characteristic changes taking place in the composition of the ash were an increase in the sodium and chlorin content, a corresponding decrease in the potassium content, and in most instances a decrease in the calcium and phosphoric acid content. In all probability a determination of the chlorin content of the milk, respectively in the ash, will furnish a clue as to whether the milk in question is of pathological origin or not.

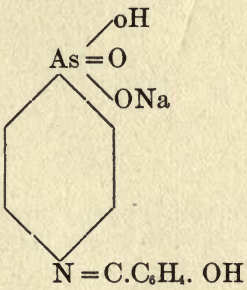
THE ARYL ARSONATES: THEIR PHARMACOLOGY CONSIDERED FROM THE EXPERIMENTAL AND PRACTICAL STANDPOINTS

By J. M. Fortescue-Brickdale, M.A., M.D. Oxon

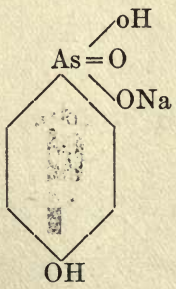
Assistant Physician to the Royal Infirmary, Bristol; Clinical Lecturer and Director of the Public Health Laboratory, University of Bristol

The fact that atoxyl (sodium *para*-amido-phenyl arsenic acid) can cause trypanosomes to disappear from the peripheral circulation is now generally known. Since the introduction of this substance by Thomas and Breinl in 1905[1] a number of allied substances have also been employed both on experimental animals and in practice. Breinl and Nierenstein[2] found that the following bodies had no trypanocidal action in infected animals:

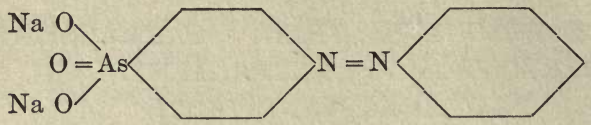
(1) Salicyl atoxyl



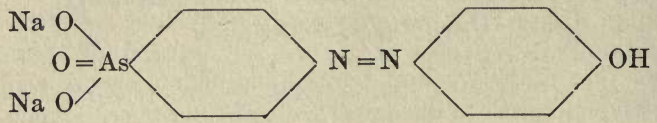
(2) Sodium *para*-hydroxy-phenyl arsenate



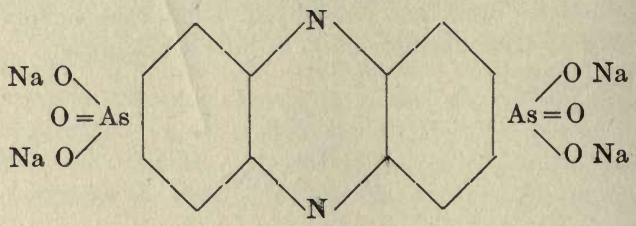
(3) Di-sodium azobenzene 4-arsenate



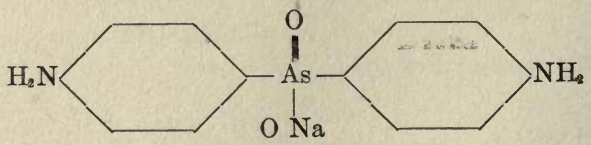
(4) Di-sodium 4-oxy-azobenzene 4-arsenate



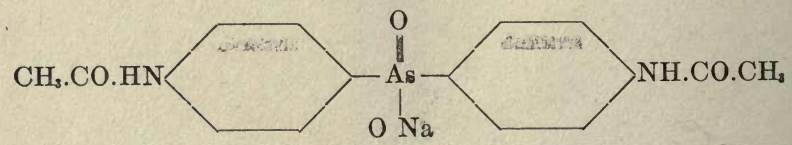
(5) Tetra-sodium phenazine 4-arsenate



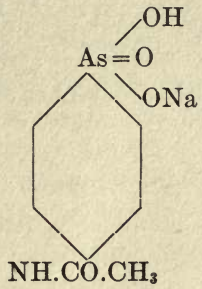
(6) Sodium di-*para*-amido-phenyl arsenate



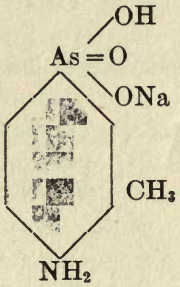
(7) Sodium di-*para*-acetyl-amido-phenyl arsenate



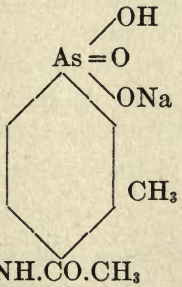
On the other hand (1) Acetyl-atoxyl



(2) Sodium 3-methyl-4-amido-phenyl arsenate (Kharsin)



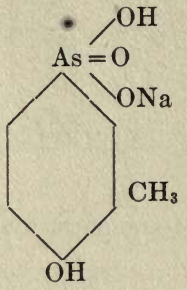
(3) Sodium 3-methyl-4-acetyl-amido-phenyl arsenate (Orsudan)



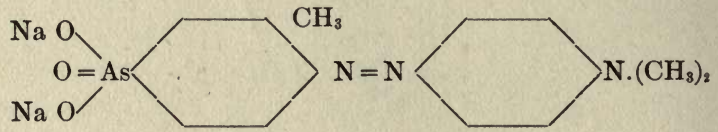
were trypanocidal for certain trypanosomes in experimental animals.

The following derivatives of Orsudan corresponding with the inactive atoxyl derivatives above enumerated had very slight trypanocidal action in the case of the first two, and none at all in the case of the two last in the list.

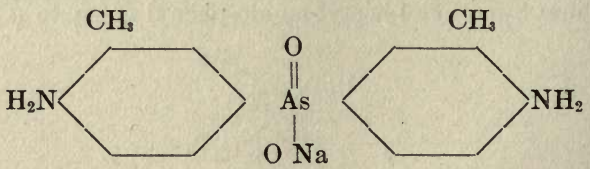
(1) Sodium 3-methyl-4-hydroxy-phenyl arsenate



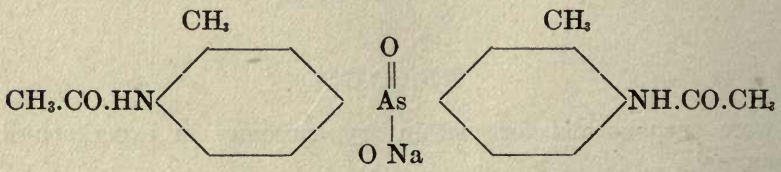
(2) Di-sodium 4-di-methylamido-2-methyl-azo-benzene 4-arsenate



(3) Sodium di-3-methyl-4-amido-phenyl arsenate



(4) Sodium di-3-methyl-4-acetyl-amido-phenyl arsenate



The acetylated compounds were found, generally speaking, to be less toxic. Acetyl-atoxyl is less toxic for animals highly susceptible to atoxyl; orsudan experimentally was without action on *T. Brucei*, but active against *T. Equiperdum* and *T. Gambiense*. Moore, Nierenstein and Todd[3] found that acetyl-atoxyl was of more value in dogs, guinea-pigs and mice infected with *T. Brucei* than the parent substance atoxyl. Salmon[4] came to a similar conclusion with regard to monkeys, fowls and rats.

Relation of chemical structures to physiological action.

An important factor in the production of a trypanocidal effect appears to be the amido group. Mesnil and Nicolle[5] and Moore, Nierenstein and Todd[6] have drawn attention to this, and the latter observers have shown a parallel phenomenon in the case of trypanocidal colouring matters.

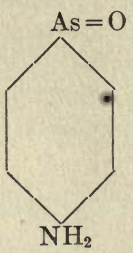
Nierenstein[7] showed that in test tube experiments, mixtures of animal serum and solutions of arsenic compounds which contain the amido group form chemical combinations, whereas in similar mixtures in which arsenic compounds without amido groups are used, no combination with serum proteins occurs.

The substances employed in his experiments were atoxyl, mono-acetyl-atoxyl and mono-benzoyl-atoxyl containing the amido group, and sodium arsenate, acetyl-benzoyl-atoxyl and sodium *para*-hydroxy-phenyl acetate, in which the amido group was either absent or substituted in respect of both the hydrogen atoms. He suggests that the amido group plays the same part as the chromogen group in a dye. The action of this group is apparently in accord with the theory put forward by Loew[8] of the interaction between amido groups with labile aldehyde groups in the living protoplasmic molecule.

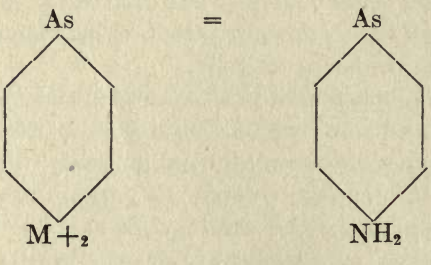
Chemical Changes in the Organism

The chemical changes in the molecule of atoxyl which take place after it has been introduced into the animal body have been variously stated. Ehrlich[9] noting the fact that atoxyl *in vitro*

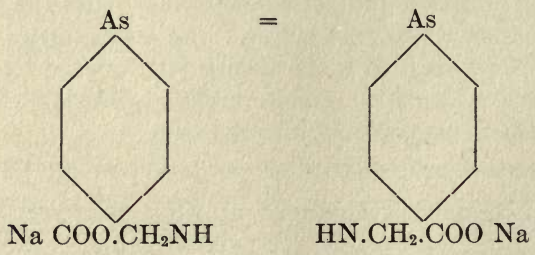
has no trypanocidal action, supposed that in the organism it was changed into a more toxic body. Two reduction products, *para*-amido-phenyl arsenic oxide



and di-amido-arseno-benzol



are trypanocidal *in vitro*, and he assumes that in the protoplasm of the trypanosome a receptor group exists, having a special affinity for the trivalent arsenic. A substitution product of diamido-arseno-benzol, namely arseno-phenyl-glycine



was prepared by Bertheim, and has been somewhat extensively used in experimental trypanosomiasis. Röhl[10] found that it was not only trypanocidal in various animals, but could be used

prophylactically in mice. It was also found to destroy *T. Lewisi*, which are not affected by other arsenic preparations. The success of this preparation, however, is dependent on the species of animal employed, and in large animals, such as donkeys, doses approaching the lethal amount could not arrest the infection. In dogs it is excreted almost quantitatively in the urine, and poisonous doses produce a marked increase in the fat and lecithin content of the blood.[11]

Levaditi and Yamanouchi[12] found that mixtures of liver emulsion, lung emulsion, or muscle emulsion with solutions of atoxyl, after incubation at 38° C. for two hours were powerfully trypanocidal. They explained this by supposing that the protein combined with the reduced arsenic, and enabled it to act on the trypanosomes after the manner of an amboceptor. Friedberger[13] supposed that the SH group in the protein molecule was the reducing agent, and obtained an analogous reaction with thioglycolic acid, which, when added to atoxyl, produced a trypanocidal substance *in vitro*. Other observers, however, have failed to confirm entirely the results of Levaditi and Yamanouchi.

Uhlnhut and Woithe[14] in 1908 obtained negative results, and Breinl and Nierenstein only occasionally got positive results when carefully repeating the original experiments. Holmes, after repeated experiments, came to the conclusion that no action such as that described by Levaditi and Yamanouchi takes place.

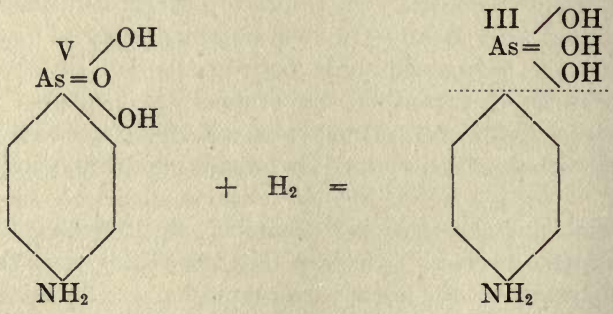
Breinl and Nierenstein,[15] however, in repeating Levaditi's experiments, were able to show that it was only when the filtrate or dialyzate of the atoxyl-liver-emulsion contained inorganic arsenic that a trypanocidal effect was obtained. Further, they showed that peroxide of Hydrogen and oxidases obtained from the liver and from vegetable sources, such as black tea, were the active agents in the production of inorganic arsenic from solutions of atoxyl. Tendron[16] and Wedemann[17] have confirmed Nierenstein's experiments by finding inorganic arsenic in the urine after the administration of atoxyl.

In contradistinction, therefore, to the theory of Ehrlich that the trypanocidal action of atoxyl *in vitro* is due to the production of a reduced trivalent arsenic compound, Breinl and Nierenstein

believe that an oxidation of the organic substance occurs, leading to the formation of inorganic arsenic, to which the effect on trypanosomes is due.

Holmes[18] states that his experiments all point to the view that a small amount of cleavage occurs, and that the therapeutic effect is due to the presence of free arsenic. Breinl and Nierenstein also found that a fermentative process occured by which atoxyl was split up into trivalent arsenic and aniline. *In vitro* this did not lead to the production of a trypanocidal substance, unless the amount of trivalent arsenic split off was sufficient to inhibit the action of the reductase.

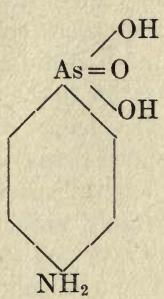
Stated fully, their view is that atoxyl when it enters the animal body is partly combined with the serum proteins by means of the amido group. This combination is then oxidized by ferment action, and the nascent inorganic arsenic exerts a trypanocidal action. At the same time, another portion of the atoxyl is reduced; the aniline is destroyed and the trivalent arsenic excreted in the faeces.



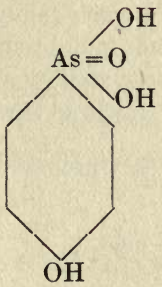
In support of this view, the following experimental evidence has been adduced, in addition to that already stated. (a) Nierenstein[19] showed that when atoxyl, mono-acetyl-atoxyl and mono-benzoyl-atoxyl were injected into rabbits and donkeys, arsenic could be detected in the serum. On the other hand, sodium arsenate and sodium *para*-hydroxyphenyl arsenate produced no arsenic in the blood serum. Acetyl-benzoyl-atoxyl, however, gave rise to arsenic in the serum, as the acetyl group is saponified and a mono-benzoyl-atoxyl is produced. About 80% of the

arsenic could be recovered from the urine, and about 40% from the faeces. Aniline could only be detected in the faeces of the horse and monkey.

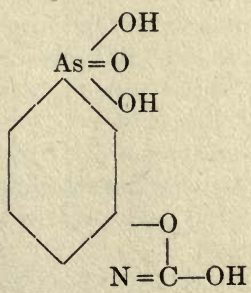
(b) The same author[20] showed that atoxyl was partly excreted in the urine as inorganic arsenic and partly as *para*-amido-phenyl arsenic acid



para-oxyphenyl arsenic acid



Oxy-carbamido-phenyl arsenic acid



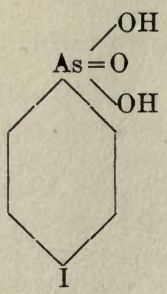
This is in accordance with the theory that atoxyl undergoes an oxidation process in the organism; a similar oxidation of Toluidine into oxycarbamido-cresol has been shown to take place.[20]

(c) Durham,[22] in 1908, published some observations on the colour of the blood in animals in the late stages of infection with *T. Brucei*. He noticed that the blood, instead of being bright red was of a "dull purplish or chocolate colour." Even after exposure to the air for a week or more, it did not regain its normal colour. He suggested that this was due to the presence of methaemoglobin and was analogous to the change in colour produced in some animals' blood by poisonous doses of dinitrobenzol. Naus and Yorke[23] have shown by careful spectroscopic examination that such blood contains partially reduced haemoglobin. Further, they were able to demonstrate that suspensions of living trypanosomes had a reducing action on haemoglobin and methylene blue, and that the incubation of living trypanosomes in the absence of air in normal defibrinated blood caused a reduction or disappearance of the oxygen combined with the haemoglobin. The carbon dioxide was not found to be correspondingly increased.

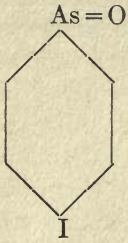
If therefore atoxyl is activated by a reduction process, it should be trypanocidal *in vitro*, which, as is well known, is not the case.[24]

(d) Mameli and Patta[25] prepared the following iodo compounds:—

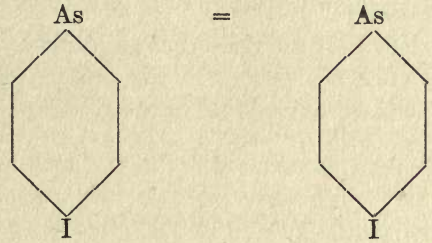
p-Iodo-phenyl arsenic acid



p-Iodo-phenyl arsenic oxide



p-di-iodo-arsenobenzol



Owing to the absence of the amido group, none of these bodies acted on *T. Brucei* either *in vivo* or *in vitro*. In the two latter, the arsenic is trivalent, and according to the reduction theory should exert a trypanocidal action.

Resistant Strains

It has been shown by Ehrlich, and is well recognized, that trypanosomes can be rendered resistant to the action of atoxyl and other drugs when these are injected into an infected animal, and that this resistance in almost all cases is specific, or holds good only for the particular drug used. It has however also been shown by Mesnil and Brimont,[26] Breinl and Nierenstein,[27] and Röhl,[28] that to a certain degree this resistance is also specific for the species of animal employed, and that atoxyl-fast trypanosomes from donkeys, for instance, lose their resistance when injected into rats.

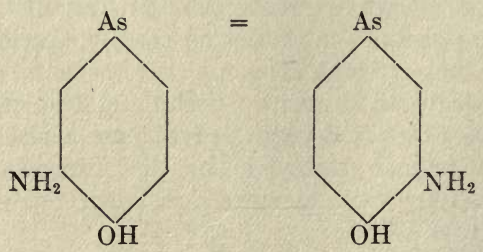
Ehrlich explains this by assuming a decreased avidity of the arseno-receptors of the trypanosomes, but strains resistant to atoxyl have been found to be influenced by arseno-phenyl-glycine and by acetyl-atoxyl. Ehrlich supposes that in the former case, the arseno receptors are not entirely put out of action, and in the latter, that there are present also acetyl receptors capable of linking the organic arsenic compounds to the trypanosomes. In fact, in his view, a number of receptors may exist in the trypanosome capable of linking it to numerous radicles.

The measure of acceptance which this hypothetical explanation gains will depend upon the importance which may be attached to bringing all the experimental results into correspondence with the " side-chain theory."

But it seems clear that the resistance acquired by the trypanosomes is a resistance not purely to the atoxyl (or other drug employed), but to the atoxyl-serum of the given species employed in the experiment.

Practical Results

The arylarsonates have been employed in the treatment of various diseases caused by Trypanosomes, both alone and in combination with other drugs. They have also been extensively employed in the spirochaetal infections, the spirochaetes, as a biological group, being held intermediate between the obviously protozoan trypanosomes on the one hand, and the bacteria—which are regarded as vegetable organisms—on the other. With regard to spirochaetes, however, recent work has been mainly concerned with the investigation of *p*-dioxy-*m*.-di-amido-benzol or salvarsan.



which has obtained a dominant position among the arsenic compounds used in the treatment of syphilis.

To deal with the practical results obtained by this preparation, alone requires a large volume; it is possible, however, and may not be without interest briefly to summarize here the position which the earlier drugs have attained as remedies for various forms of trypanosome infection in man and animals.

The most important pathogenic varieties of trypanosomes are *T. Brucei*, causing nagana or tsetse fly disease in cattle and other animals; *T. Gambiense* causing " sleeping sickness " in man; and *T. Evansi* causing " surra " in horses and other animals.

(1) *T. Brucei.* Numerous experimental results have been obtained with this organism, as it multiplies rapidly in the blood of small laboratory animals and is thus a convenient member of the group for observation. The immediate effect of the injection of atoxyl is almost always favourable, but the trypanosomes recur in the blood after a longer or shorter interval in spite of repeated injections and very few permanent cures have been obtained. In practice, and also experimentally, better results have been obtained by the combination of atoxyl with various dyes or with mercury salts. The theoretical explanation of this fact appears to be either that a certain number of trypanosomes which have been subjected to the atoxyl treatment survive and become immune to the drug, but not to some other drug, such as trypan-red or mercury; or that in the organism developmental forms of trypanosome occur which are not affected by arsenic compounds, but which are susceptible to other trypanocides.

(2) *T. Gambiense.* The long course of the infection by this parasite makes observations on the ultimate effect of drugs very difficult. In spite of earlier reports of successes by means of atoxyl in human beings, some authorities doubt whether a case has ever been cured. Mott,[29] who has recently reviewed the whole question, inclines to the view that possibly, if the diseases is treated before the organisms have invaded the subarachnoid space, a cure can take place. He describes a *post-mortem* on a case which had been very energetically treated with atoxyl, and in which there was no evidence of involvement of the central nervous system. Death had occurred from intercurrent disease

of another character. The difficulty appears to be that it is at present impossible to determine at what point, in the clinical course of the infection, the parasites (or their toxins) have already caused such damage to the nervous structures that the lesions are likely to be progressive, even if all the trypanosomes have been destroyed. Mott quotes the opinion of Hodges, based upon the observation of over 5000 cases, that the course of the disease is undoubtedly modified, if not cut short, by the administration of atoxyl and its derivatives.

(3) *T. Evansi.* Many authors have reported a certain percentage of successes in the treatment of surra in horses and mules with various forms of arsenic, sometimes combined with other drugs. The most successful results appear to have been obtained by a combination of atoxyl subcutaneously with arsenious acid by the mouth. The atoxyl has the effect of rapidly causing the trypanosomes to disappear from the peripheral circulation, and is the best method of introducing arsenic by injections, as it causes no local lesions. The continued exhibition of arsenic which is necessary in these cases can be conveniently and more effectively carried out by the inorganic form of arsenic given by the mouth. In some cases, more than 70% of animals naturally infected have been permanently cured by this method.

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(*Abstract*)

THE UTILIZATION OF INGESTED PROTEIN AS INFLUENCED BY UNDERMASTICATION ("BOLTING") AND OVERMASTICATION ("FLETCHERIZING")

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and P. B. Hawk

Jefferson Medical School, Philadelphia

The subjects of the investigation were two young men (laboratory assistants) weighing 63.0 kg. (J) and 58.3 kg. (F) respectively at the commencement of the experiments. The study was divided into four periods as follows, each period being seven days in length: (1) normal, (2) bolting, (3) Fletcherizing, (4) normal. A uniform diet was fed each subject throughout the course of the test. The diets as fed were as follows:

Subject "F"			Subject "J"		
Meat	185	11.21	Meat	215	13.03
Graham Crackers	150	1.93	Graham Crackers	150	1.93
Milk	650	3.31	Milk	800	4.07
Butter	150	0,16	Butter	150	0.16
Water	1800	——	Water	2100	——
Sodium chloride	1.6	——	Sodium chloride	2	——
Agar-agar	10	——	Agar-agar	15	——
Total		16.60	Total		19.18

It will be noted that meat contributed the major part of the nitrogenous portion of the diet. This meat consisted of the best "round steak" procurable, which was freed from all visible fat and connective tissue. It was then cut into approximately fifteen-millimeter cubes and cooked by being allowed to "simmer" in boiling water for two and one-half hours. After being thoroughly mixed and sampled for analysis it was placed in pint

Mason jars and sterilized at 125° C. Agar-agar was included in the diet to facilitate defecation.

During the preliminary period of the experiment the food was masticated *normally*; in the period of undermastication the diet was " bolted " with no attempt at mastication; in the period of Fletcherism the food was chewed until carried down the oesophagus by the " swallowing impulse " and in the final period normal mastication was again practiced.

Total nitrogen (Kjeldahl) determinations were made on foods, feces and urine. The feces were " separated " by means of carmine, and satisfactory differentiations were secured in every instance. All stools were examined *fresh. Microscopical meat residues of varying sizes were found in every stool passed during the bolting periods.* The weights of these residues in one particular stool aggregated nearly *seventeen grams.*

An attempt was made to secure data as to the actual daily output of " metabolic nitrogen " by the subjects of the experiment. To this end the suggestion of Mendel and Fine was adopted. A *nitrogen-free* diet of similar energy value to the experimental diet was ingested, agar-agar being added in sufficient quantity to bring the daily fecal output up to the level of the fecal output of the experiment proper. This diet was fed each subject for a period of four days and the average output of fecal nitrogen for the final three days of the period was taken as the " metabolid nitrogen check." This correction was then applied to the fecal nitrogen values obtained in the four periods of the experiment proper. The utilization values *corrected for metabolic nitrogen* are as follows:

Period	Utilization per cent.	
	F	J
Normal	97.0	95.5
Bolting	95.4	95.7
Fletcherizing	97.2	97.5
Normal	97.0	97.5

The data for F indicate that the protein of the diet was somewhat less efficiently utilized by this subject during the period of food bolting than during the periods of normal mastication and

Fletcherism. The uniformity of the values for the two periods in which the food was normally masticated and the practical duplication of this value when the subject Fletcherized are points to be emphasized.

In the case of J the protein portion of the diet was fully as satisfactorily utilized when bolted as when normal mastication was practiced. As he passed from bolting to Fletcherizing there was rather better utilization as is shown in the table. The improved utilization in the period of Fletcherism continued throughout the following normal period, a fact which may perhaps be interpreted as due in part to the influence of the preceding period of hypermastication. It should be mentioned in this connection, however, that the utilization value for the normal period of this subject (95.5%) is rather lower than one would expect when it is recalled that the "metabolic product" correction has been applied. Throughout the course of this normal period J was in a continual state of worry and it is quite possible that his digestive efficiency was lowered somewhat from the normal. In the bolting period he was more normal so far as mental attitude was concerned. We are inclined to believe that the utilization values for the bolting, Fletcherizing and final normal periods are dependable values whereas the value for the preliminary period of this subject we believe to have been influenced by non-experimental conditions.

Our data indicate that when meat is bolted in fifteen-millimeter cubes it is somewhat less efficiently utilized than when normally masticated or Fletcherized. However, the difference in utilization is not pronounced and cannot be considered as furnishing an experimental basis for the belief that food bolting is harmful to the organism. The protein portion of the diet was no more efficiently utilized when the food was chewed until carried down the oesophagus by the "swallowing impulse" than when it was masticated in a normal manner. In other words our data fail to show the advantages of Fletcherism or the harmfulness of food bolting.

(*Extrait*)

CHLORURE DE L'ACIDE DICHLOROARSINOBENZOIQUE. ETHERS DES ACIDES BENZARSINEUX ET BENZARSINIQUE

PAR MM. E. FOURNEAU ET K. ÖCHSLIN

Paris, France

Les auteurs ont préparé à l'état de pureté le chlorure de l'acide dichlorarsinobenzoique dont la préparation avait déjà été tentée par LaCoste. Ce chlorure est distillable sans décomposition dans le vide et bout vers 189-190° sous 19 mm. C'est un liquide sirupeux qui cristallise spontanèment au bout de plusieurs semaines et immédiatement par a orcage. Il est soluble dans l'éther, le chloroforme, etc. Il se comporte comme le chlorure de benzoyle envers les alcools les phénols les aminoalcools et la quinine, etc. Les auteurs ont ainsi prémaré 1° léther benzarsinique de la quinine, soluble, a la fois, dans les alcalis et les acides en donnant des solutions insipides; 2° le produit de réduction de l'éther benzarsinique ou benzars énoquinine; 3° l'éther du gayacol et 4°, l'arsénostovaine.

Ce dernier produit est un anesthésique local don les fonctions déterminant l'anesthésie entrainent la chaine arsénicale. Il s'agit la peurent les aideurs, du premier essai rationnel de transport d'une substance active dans un tissus désigné l'avance, qui, dans le ces particulier et la substance nerveuse.

ETUDE COMPARÉE DES PRÉSURES DE L'AMANITE PHALLOIDE ET DE L'AMADOUVIER—RELATIONS ENTRE LES PRÉSURES DES BASIDIOMYCÈTES ET DES VÉGÉTAUX SUPÉRIEURS

Note de M. C. Gerber

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Les sucs de ces deux champignons sont des coagulants énergiques du lait.

Cette coagulation est une caséification diastasique. Les agents de la caséification sont très différents dans les deux espèces quant à leur localisation, leur résistance à la chaleur, leur action sur les laits cru et bouilli, l'influence de certains sels sur leur fonctionnement.

A. Localisation. A l'opposé de ce que l'on observe avec les autres *Agaricacées*, la présure de l'Amanite phalloïde (amanita phalloida Fr) est beaucoup moins abondante dans les lames sporifères que dans la partie stérile du chapeau. Au contraire, conformément àce que l'on observe avec les autres *Aphyllophoracées* la présure de l'Amadouvier (*Fomes fomentarius* Fr) est beaucoup plus abondante dans les tubes sporifères que dans le reste du chapeau. Ce champignon d'ailleurs constitue le matériel de choix pour établir la relation étroite qui existe entre la formation des spores et l'activité présurante du suc, chez les Basidiomycètes. Chez ce Porohydné, en effet, les tubes hyméniaux naissent par poussées successives et d'une façon telle qu'ils constituent des couches superposées très distinctes, la couche la plus ancienne, qui a perdu ses spores étant celle qui est fixée directement au chapeau, et la plus jeune qui est en voie de sporulation étant au contraire celle qui termine, en bas, la série verticale des couches superposées. Sur un amadouvier possédant trois couches de tubes, nous avonsséparé (ce qui est très facile) celles-ci les unes des autres et en avons extrait séparément les sucs. Nous avons constaté que l'activité présurante du suc de la couche la plus jeune, sporifère, étant dix, celle de la couche moyenne, est 5 et

celle de la couche la plus âgée qui a essaimé presque toutes ses spores, 2, 5 seulement: Cette dernière est même deux fois moins forte que l'activité présurante du suc rétiré de la partie stérile du chapeau qui fournit l'amadou.

B. RESISTANCE A LA CHALEUR. La présure de *Amanita phalloïdes* Fr est moins résistante à la chaleur que celle de *Fomes fomentarius* Fr. La première est, en effet, complètement détruite par un séjour de 5 minutes à 65°, alors que la seconde ne perd toute action présurante qu'après un pareil temps de chauffe à 75°:

C. ACTION SUR LES LAITS CRU ET BOUILLI. A fortes doses, et par suite dans le cas des caséifications rapides, les deux présures coagulent plus rapidement le lait cru que bouilli. A faibles doses, et par suite dans le cas des caséifications lentes, la présure de la phalloïde seule continue à coaguler plus rapidement le premier liquide que le second; celle de l'Amadouvier, au contraire, coagule plus lentement le lait cru que bouilli.

Cette différence est due à l'action favorisante du calcium plus prononcée dans le cas de la première diastase et à l'action empêchante des albumines et globulines du lait cru plus énergique dans le cas de la seconde.

Quelques Basidiomycètes appartemant surtout au groupe des porohydnées et qui supportent de grandes différences de température se comportent comme l'amadouvier, leurs présures coagulent mieux, à faibles doses le lait bouilli pur que le lait cru pur, elles sont dites présures du lait bouilli. Un plus grand nombre, qui ne peuvent vivre qu'entre des limites assez étroites de température, se comportent comme l'Amanite phalloïde; leurs présures à toutes doses, coagulent mieux le lait cru pur que le lait bouilli pur; elles sont dites présures du lait cru; quelques unes, plus calciphiles, sont incapables de coaguler le lait bouilli pur (*Pleurotus ostréatus* L *Armillaria caligata Viv, Clitocybe inversa Scop*); certaines mêmes (*Hypholoma sublateritium Betc*) ont un tel besoin de calcium que la quantité de cet élément dissous dans le lait cru lui-même est insuffisante pour leur permettre d'en mener à bien la caséification; elles n'agissent que sur le alit calcifié soit directement, (addition de $CaCl_2$) soit indirectement (addition de quelques molécules milligr. HCl dissolvant le phosphate de chaux en suspension). Nous les faisons néanmoins entrer

dans le type *présures du lait cru* dont elles possèdent tous les autres caractères.

D. Influence de Certains Sels sur la Caseification. Les chromates des métaux alcalins, les sels neutres de Nickel, de Cobalt, de Zinc, de Cadmium, de Cuivre, d'Argent et surtout ceux de Mercure d'Or et des métaux du groupe du Platine, ajoutés au lait empêchent ou retardent fortement la caséification par les sucs d'Amadouvier et des autres basidiomycètes appartenant au groupe *Présures du lait bouilli*; au contraire ils retardent faiblement, ou pas du tout, ou même accélèrent la caséification par les sucs de l'Amanite phalloïde et des autres basidiomycètes appartenant au groupe *Présures du lait cru.*

Les corps retardateurs: Lactoglobulini, lactalbumines et sels pécédents agissent non pas sur la diastase qu'ils rendraient moins active, mais sur la caséine qu'ils rendent plus résistants en formant avec elle, très probablement un complexe difficilement dédoublable.

E. Parallelisme Entre les Presures des Basidiomycetes et Celles des Vegetaux Superieurs. Nous avons montré dans des travaux antérieurs, que les sucs présurants des végétaux supérieurs se groupent en deux classes correspondantes à celles que nous venons d'établir.

La première classe, de beaucoup la plus importante dont le type est la présure du *Vasconcelba Quercifolia* correspond à celle de l'Amadouvier; les ferments protéolytiques qu'el'e renferme sont en effet, très résistantes à la chaleur, coagulent de préférence le lait bouilli et voient leur action caséifiante fortement entravée par les sels des électrolytes ci-dessus. La seconde classe, moins grande, dont le type est la présure du murier à papier (*Brounonétia papyrifera* L), correspond à celle du type Amanite phalloïde; les ferments protéolytiques qu'elle renferme sont généralement peu résistants à la chaleur—Le murier à papier fait exception—coagulent de préférence le lait cru et voient leur action caséifiante peu ou pas influencée par les sels précedents, parfois même favorisée.

Il y a donc parallélisne entre les présures des Basidiomycètes et celles des végétaux supérieurs; mais les premières sont en grande majorité des présures du lait cru et lessecondes, le plus souvent, des présures du lait bouilli.

SCHNELLES VERFAHREN ZUR BESTIMMUNG DER HARNSÄURE IM HARN

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Die Bestimmung der Harnsäure im Harne ist in vielen Fällen für medizinische Zwecke von Wichtigkeit. Es ist wünschenswert in manchen Krankheiten öfters die Menge dieser, im Harne ausgeschiedenen Säure zu kontrolieren. Es kommen demnach für klinische Zwecke diejenige Verfahren in Betracht, welche bei schneller und leichten Ausführung genügend richtige Ergebnisse liefern. Von den bestehenden Verfahren zur Bestimmung der Harnsäure sind als mehr oder minder genau bekannt die Metoden von Ludwig, Hopkins, Jolles und ihre zahlreichen Modificationen und Verbesserungen. Sämmtliche diese Verfahren erfordern aber mehr oder weniger Zeit zu ihrer Durchführung und Bereitung besonderer Lösungen.

Mit der Mehrazhl dieser Metoden habe ich auch entsprechende Vergleichsversuche angestellt ohne übereinstimmende Zahlen zu erhalten, manche Verfahren lieferten sogar ganz falsche Ergenhnisse.

Ich bemühte mich deshalb, da ich grössere Anzahl solcher Bestimmungen durchzuführen hatte, eine andere, möglichst rasche u. leichte, dabei aber genügend genaue Metode auszuarbeiten.

Dies gelang mir auf Grund des Prinzipes der Hopkinsschen Metode: Ausscheidung von harnsaurem Ammoniak und durch weitere einfache Manipulation mit demselben. Mein Verfahren basiert nämlich auf der direkten acidimetrischen Titration des ausgeschiedenen und ausgewaschenen Ammoniumurates. Die Harnsäure, als sehr schwache organische Säure lässt sich leicht durch stärkere Mineralsäuren aus ihren Salzen frei zu machen. Die Mineralsäuren bilden mit der betreffenden Base, mit welcher die Harnsäure verbunden war, entsprechende Salze. Der

Uiberschuss der Mineralsäure wird durch entsprechende Indicatoren genau angezeigt. Als Indicator hiezu eignet sich besonders Methylorange, welche bekanntlich durch Uiberschuss von Mineralsäure rot und nach Neutralisation mit Lauge wieder citronengelb sich färbt. Zur Titration eignet sich am besten 1/10 n Schwefelsäure. Das Verfahren empfiehlt sich wie folgt durchzuführen.

Zur Untersuchung werden 50-100 cc Harn in ein Becherglas abgemessen, 18 resp. 36 g reines pulveriges Ammoniumchlorid zugesetzt und bis zur Auflösung desselben gerührt. Nach einer halben Stunde kann schon durch Papierfilter filtrirt werden. Nach vollständigem Abfliessen der Mutterlauge wird dreimal nach einander mit gesätigter Salmiaklösung ausgewaschen und zwar am besten, dass jedesmal das Filter mit der Waschflüssigkeit voll gefüllt und vollständig abfliessen gelassen wird.

Der Niederschlag sammt Filter wird sodann in das Becherglas, in welchem der Niederschlag gebildet wurde, gegeben, ca 50 cc destilirtes Wasser zugesetzt, zum Kochen erhitzt und 2 Tropfen Methylorangelösung zugesetzt, damit nur schwache Gelbfärbung entsteht. Mehr Tropfen von diesem Indicator zuzusetzen ist zu vermeiden, da hiebei eine rotgelbe Färbung entsteht, welche die Erkennung des Uiberganges der Reaktion sehr erschwert. Nachher wird 1/10 n Schwefelsäure im Uiberschuss bis zur kenntlichen Rotfärbung zugesetzt und mit 1/10 n Lauge bis zur Eintretung der gelben Färbung zurücktitriert. Zur Gewinnung gehöriger Geläufigkeit in Erkennung dieses Farbenumschlages und Gewinnung vollständig sichtiger Resultate empfiehlt es sich nochmals die Säure bis zur Rotfärbung zusetzen und mit der Lauge zurücktitrieren.

Die verbrauchten cc der 1/10 n Säure, multipliciert mit dem Faktor 0.01682 ergeben die Menge der Harnsäure in g in der abgemessenen Harnmenge.

Ist der untersuchte Harn nicht mehr klar, muss der eventuell ausgeschiedene harnsäurehältige Niederschlag in Lösung gebracht und die ausgeschiedenen Phosphate abfiltrirt werden. Hierbei wird wie folgt verfahren: Nach gehörigem Durchmischen werden 100 cc Harn in Becherglas abgemessen, einige Tropfen Phenolphtaleinlösung zugesetzt, erwärmt und tropfenweise Aetznatron bis zur dauernden Rotfärbung zugesetzt, eine Weile gekocht,

damit ein Teil der Lösung verdampft und alles in ein 100 cc Kölbchen gebracht und mit destillirtem Wasser nach Abkühlung zu 100 cc nachgefüllt werden kann. Sonach wird durch trockenes Filter filtrirt und vom Filtrate 50 cc zur Bestimmung der Harnsäure nach der oben angegebenen Metode entnommen.

Mit diesem Verfahren wurden zahlreiche Kontrolversuche sowie im Harne als auch in reinen Harnsäurelösungen ausgeführt, welche sehr befriedigende Resultate ergeben haben. Da die käufliche reine Harnsäure zu diesen Versuchen nicht genügend rein war, wurde dieselbe zu diesen Versuchen wie folgt gereinigt: 1 g der käuflichen Harnsäure wurde in 50 cc Wasser suspendirt, einige Tropfen Phenolphtaleinlösung zugesetzt, erwärmt und tropfenweise Aetznatron bis zur dauernden Rotfärbung zugesetzt, welches wieder durch ein Tropfen Salzsäure entfärbt wurde. Nachher wurde die Flüssigkeit filtrirt und das reine Filtrat nach Zusatz von 2 Tropfen Methylorangelösung durch einen kleinen Uiberschuss von konzentrirter Salzsäure (bis zur eintretenden Rotfärbung) zersetzt, die ausgeschiedene Harnsäure durch das Ludwigsche Filtrationsrohr abfiltrirt, mit destillirtem Wasser zum vollständigen Verschwinden der Chlorreaktion ausgewaschen, bei 40° C. im Wassertrockenschrank und nachher über Schwefelsäure zur Gewichtskonstanz ausgetrocknet. Auf diese Weise gereinigte Harnsäure wurde zu den Kontrolversuchen verwendet. Zu diesem Zwecke wurde eine bestimmte Menge Harnsäure abgewogen, in destillirtem Wasser suspendirt, mit titrirter Aetzlauge unter Zusatz von Phenolphtalein neutralisirt, auf bestimmtes Volumen gebracht und von dieser Lösung immer nötige Mengen entnommen um nach Zusatz von bestimmter Menge destillirten Wassers den im Harne vorkommenden Harnsäuremengen entsprechende Lösungen zu bekommen.

I

Versuche mit unreiner Harnsäure (in 100 cc):

1. Verwendet 0.10 g, gefunden 0.0841 g
2. Verwendet 0.02 g, gefunden 0.0168 g
3. Verwendet 0.10 g, gefunden 0.0841 g

II

Versuche mit gereinigter Harnsäure (in 100 cc):

4. Verwendet 0.04 g, gefunden 0.0390 g
5. Verwendet 0.08 g, gefunden 0.0782 g
6. Verwendet 0.20 g, gefunden 0.1952 g
7. Verwendet 0.20 g, gefunden 0.1952 g
8. Verwendet 0.30 g, gefunden 0.2938 g
9. Verwendet 0.01 g, gefunden 0.0097 g

Versuche mit Bestimmung der Harnsäure im Harne führten zu ebenso günstigen Ergebnissen. Die Harnsäuremenge wird gewöhnlich auf 1 L oder auch auf in 24 Stunden entleerten Harn gerechnet angegeben. Da die tägliche Harnmenge öfters schwer anzugeben ist und die Berechnung auf 1 L Harn bezogen nicht einwandfrei sein kann, da sehr von der momentanen Konzentration des Harnes abhängend, dürfte einer Erwägung wert sein, ob nebstdem auch die Harnmenge auf Trockensubstanz des Harnes bezogen nicht angegeben werden sollte. Die Trockensubstanz könnte zu diesem Zwecke entweder refrektometrisch oder aus dem specifischen Gewichte nach Uiberführung in Saccharometergrade (nach der Ballingschen oder Brixschen Tabelle) bestimmt werden. Bei zuckerhältigen Harnen müsste die Harnsäuremenge auf die zuckerfreie Trockensubstanz bezogen werden. Die prozentische Harnsäuremenge in der Trockensubstanz könnte "Harnsäurequotient" benannt werden. So z.B. enthält ein Harn 0.037% Harnsäure. Das specif. Gewicht—1.0199, entsprechend 5.06% saccharometrischer Trockensubstanz. Es sind demnach in 100 T. Trockensubstanz 0.73 T. Harnsäure enthalten, oder der Harnsäurequotient—0.73%.

THE UTILIZATION OF INDIVIDUAL PROTEINS BY MAN AS INFLUENCED BY REPEATED FASTING

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A study was made of the utilization of *meat*, *milk*, *glidine*, *plasman* and *gluten*, substances of which the nitrogenous portion is made up for the most part in each instance by some particular individual protein. The utilization of a standard "cracker-milk-butter-peanut-butter" diet was studied for comparison. In order that a uniform basis for the study of the utilization might be secured the subject (58 kg. man) was fasted for a period of two days previous to the ingestion of the various experimental diets. A series of eight such fasts was made, water being taken in uniform quantity daily.

The standard diet furnished 12.12 grams of nitrogen and 2500 calories of energy. For a period of two days immediately following each fast this diet was increased 50% thus furnishing 18.18 grams of nitrogen and 3750 calories of energy. Following this increased diet the normal nitrogen and calorific level was again assumed for a three-day interval. At this point the subject again fasted for two days. In brief the experimental plan consisted of a series of two-day fasts separated by five-day feeding intervals the diet for two days immediately following the fast being increased 50% above that fed during the next three days. No attempt was made to separate the feces of the two-day fasting intervals. On the basis of previous fasting experiments in this laboratory it has been found that the output of feces by fasting men even yields approximately 0.1 gram of nitrogen per day. This correction was applied in this case.

The utilization and nitrogen balance data for the experiment are summarized below.

UTILIZATION VALUES (PER CENT.)

Diet	Days	Source of Protein Fed								
		Milk	Glidine	Standard I	Standard II	Plasmon I	Plasmon II	Standard III	Gluten	Meat
150%	2	94.2	97.5	96.5	92.2	96.3	95.8	93.8	90.5	97.4
100%	3	94.3	96.5	85.5		94.5			86.0	96.5
Nitrogen Balance for 5 days Grams N	5	+7.8	—1.3	+1.4	— —	±0.0	+5.0	— —	—9.7	+11.3

An examination of the data indicates that meat and glidine were utilized more efficiently than the other nitrogenous foods fed, whereas plasmon, milk, standard diet and gluten follow in the order given. It will be noted that in practically every instance, no matter what the character of the protein, there was more complete utilization during the two-day period of high diet immediately following the fast, than during the subsequent three-day interval on the normal nitrogen level.

From a consideration of the nitrogen balances we find that the greatest nitrogen gains were made upon meat and milk. The standard diet, plasmon I, glidine and gluten were next in order. The nitrogen balance for plasmon II is not comparable with the other nitrogen balances inasmuch as it relates solely to the two days during which the 150% diet was fed. There was of course always a pronounced retention of nitrogen during this period no matter what the character of the ingested protein. It will be observed that the most pronounced plus balances were obtained when proteins from *animal sources* (meat and milk) were fed.

The proteins of vegetable origin, e.g., glidine and gluten yielded minus balances. This speaks for the greater efficiency of the animal proteins. It is also an interesting fact that the nitrogen of milk was a much more satisfactory nutritive medium than was the dried milk protein preparation (plasmon).

The fact that utilization values are not necessarily reliable indexes of efficiency is demonstrated through the data for meat and glidine. The protein from these two sources was equally well utilized. However when we examine the nitrogen balances we observe that the meat yielded a *plus* nitrogen balance of 11.3 grams whereas glidine gave a *minus* balance of 1.3 grams. It is evident therefore that so far as digestion and absorption are concerned there is apparently no margin of choice between meat and glidine. However, when the question of the retention of their nitrogen for the use of the organism is concerned the evidence is strongly in favor of the meat.

A word of explanation should be offered regarding the low utilization values for gluten. It so happened that there was a delay in the arrival of the gluten flour and it was necessary to feed it before its composition could be determined by analysis. The nitrogen value as recently determined by Mendel and Fine (14%) was therefore made the basis of our calculations. Subsequent analyses (a dozen or more) indicated that our gluten contained *less than* 7% *of nitrogen.* Therefore instead of feeding the same quantity of nitrogen in the form of gluten as was fed in the form of the other proteins we were feeding *less than one-half as much nitrogen.* For this reason the utilization values for gluten are in no way comparable with the other utilization values. It might be well to mention the fact that we made a starch determination on the gluten flour as purchased and found 50.7% *of starch.* This particular specimen can hardly be considered a satisfactory flour for the use of diabetics.

PURINE CATABOLISM IN THE MONKEY

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While the urine of man contains considerable amounts of uric acid with almost negligible traces of allantoin, it is the latter substance which for the lower mammals forms the principal product of purine catabolism. The apparently unique position occupied in this respect by the human species makes it of interest to ascertain the fate of purine material in apes and monkeys. Our contribution to the problem is at present limited to observations upon a female guenon monkey (*Cercopithecus callitrichus*), weighing 4.7 kilograms. We have already[1] reported results showing that in this animal the allantoin-purine ratio is of the same order as in the lower mammals. The present communication deals with later experiments which confirm and amplify our earlier conclusion.

The monkey was maintained for ninety-six days on a diet of milk, peanuts, and bananas, the urine being regularly collected in two-day periods. Of the forty-eight urine samples thus obtained twenty-six represented the normal excretion on the (presumably) purine-free regime selected. The allantoin nitrogen of these twenty-six controls varied between 20 and 32 milligrams; in twenty cases it lay between 26 and 31 milligrams; the average of all was 27.7. Purine nitrogen ranged from 6.7 to 13.9 milligrams; in twenty cases from 9.1 to 12.1; average, 11.0. This purine output appeared to consist mainly of bases; at any rate uric acid was never isolated from the normal urine. Of the total allantoin-purine nitrogen of individual samples allantoin accounted for a minimum of 64 and a maximum of 82 per cent. Each of these extremes was exceptional ; on all but six occasions the ratio lay between 71 and 76; its mean value was 72.

[1]Proc. Amer. Soc. Biol. Chem., II, p. 73, 1912; Journ. Biol. Chem., XI, p. xxxix.

The results of the oral or subcutaneous administration of Sodium nucleate, sodium urate, and allantoin are summarized in the table. (The figures are milligrams of nitrogen; for sodium nucleate, of which 2 gram doses were given they represent nitrogen of the purine ring only.)

Substance	Amount given	Method	Amount recovered		Total	Percentage recovered
			Uric acid	Allantoin		
Sodium nucleate	140	oral	3.5	12.7	16.2	12
	140	oral	5.6	30.1	35.7	26
Sodium urate	32.9	oral	9.3	0.0	9.3	28
	34.4	oral	0.0	1.4	1.4	5
	65.9	oral	1.2	4.6	5.8	9
	66.6	subcut.	28.9	37.6	66.5	100
Allantoin	45.8	oral		0.0		0
	96.3	oral		22.3		23
	98.0	oral		13.5		14
	33.5	subcut.		25.1		75
	34.1	subcut.		27.9		82
	70.4	subcut.		63.1		90

The feeding experiments with nucleic acid demonstrate the conversion of the purine nuclei into allantoin with uric acid as an intermediate product. The proportion in which these appear is such as might have been expected, allantoin accounting for 78 and 84 per cent. of the recovered purine nitrogen. The total amount of the latter is however but a fraction of that administered. The result of feeding uric acid is even less illuminating; very little indeed reappears in the urine, and the allantoin excretion is not appreciably affected. How the deficit in either case is to be explained—whether by destruction in the intestine, or by failure of absorption—we have not yet been able to decide.

One must of course reckon with the possibility of allantoin being not a terminal but an intermediate product. The small percentage of ingested allantoin recoverable in the urine would harmonize with such a view. The injection experiments seem to negative it completely. The quantity of allantoin recovered unchanged after subcutaneous introduction is practically as great as if it had been directly dissolved in the urine. Injected uric acid is likewise completely accounted for, more than half being converted into allantoin. One seems forced meanwhile to conclude that in the intermediary metabolism of the monkey allantoin is indestructible, and that it constitutes not only the principal but also the final product of purine destruction.

THE RELATION BETWEEN CHEMICAL CONSTITUTION AND PHYSIOLOGICAL ACTION AS EXEMPLIFIED BY THE GLYOXALINES, ISO-QUINOLINES AND ACID AMIDES

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At the Eighth International Congress of Applied Chemistry we discussed the relation between chemical constitution and physiological action, and entered into particulars with respect to three classes of chemical compounds, namely, the Arylarsonic Acids, the Alkamine Esters and the Tropeines. Little has been added to our knowledge of the physiological action of the two last mentioned classes of compounds since then, but much progress has been made by Ehrlich and his collaborators in the case of the organic derivatives of arsenic. Briefly, it has been shown that the arylarsonic acids yield on reduction successively arylarsenious oxides and arsenoaryls. A very large number of these compounds have been physiologically examined, and have led eventually to the production of 3:3′-diamino-4:4′-dihydroxyarsenobenzene, which has since been largely employed in the treatment of syphilis and other protozoal diseases.

The success attending the use of organic arsenic compounds has naturally led to the production and physiological examination of other organo-metalloidal and organo-metallic compounds. For instance, several investigations have been carried out with a view of preparing aryl antimony compounds for use in medicine. None of these, however, has reached a satisfactory conclusion; and this has been due partly to the physical unsuitability, such as insolubility, of many of the compounds prepared, which has hindered or prevented their physiological investigation, and

partly to the fact that such as could be satisfactorily tested failed to have the desired action.

Another development of work on these lines was suggested by the combined treatment of syphilis with sodium p-aminophenylarsonate and mercurials, or with a mercury salt of p-aminophenylarsonic acid. It was thought that the introduction of one or more mercury residues into the aromatic nucleus of phenylarsonic acids might give rise to valuable therapeutic compounds. A considerable series of oxymercury derivatives of phenylarsonic acids were therefore prepared,[1] a typical example of such compounds being disodium 3-oxymercury-4-aminophenylarsonate (HOHg.) (NH_2) C_6H_3.AsO ($ONa)_2$, which is a derivative of sodium p-amino-phenylarsonate. These compounds contain mercury in a non-ionised condition. They do not coagulate albumin, and are satisfactory on the whole as regards toxicity and suitability for hypodermic injection, but the results obtained on physiological examination have been disappointing.

In connection with organo-metalloidal compounds, von Wassermann's recent use of an eosin-selenium compound in experiments with cancerous mice has aroused widespread interest, and it will be interesting to follow the further developments of this work.

On this occasion, we propose again to deal with the relation between Chemical Constitution and Physiological Action in certain selected fields in which we have been working, namely, glyoxalines, *iso*quinolines and acid amides.

The investigation of the glyoxalines has proceeded in two directions of physiological interest dealing respectively with synthetic substances allied to 4(or 5)-β-aminoethylglyoxaline and pilocarpine. Our knowledge that aminoethylglyoxaline is a base of physiological importance is due to the extended investigations of Barger and Dale, who have shown that it is one of the active principles of ergot.

In the course of the last few years, great progress has been made in the elucidation of the bases to whose combined effect the properties of ergot are due, and it is now known that besides

[1]Wellcome and Barrowcliff (*Eng. Pat.* 12,472 of 1908).

the alkaloids ergotinine and ergotoxine, a number of other bases also contribute towards it.

These bases are derived from amino-acids by the loss of the elements of carbon dioxide, and are derivatives of ethylamine; the two most important are p-hydroxyphenylethylamine derived from tyrosine, and aminoethylglyoxaline from histidine. One of the most important and thorough examinations of the pharmacology of any series of compounds has recently been carried out by Barger and Dale in the study of the relation between chemical structure and sympathomimetic (adrenine-like) action of the phenylalkylamines and their phenolic derivatives, the class to which p-hydroxyphenylethylamine belongs.

Their results are too profuse to receive consideration in detail, but we may draw attention to certain analogies which exist between the aminoalkyl derivatives of benzene, and those of glyoxaline, which latter are more fully described in the special part of this paper.

In the case of the phenylalkylamines, the optimum structure for physiological effect is present when the benzene ring and amino-group are separated by a chain of two carbon atoms, and another optimum condition is the presence of two phenolic hydroxyls in the 3:4 positions relative to the side chain.

Similarly it has been found that only those aminoalkylglyoxalines have any pronounced physiological activity, in which the glyoxaline ring and amino-group are separated by a chain of two carbon atoms. Here again the presence of an acidic grouping in the ring is an optimum condition; in this case it is of course the imino-group which so functionates in the place of the hydroxylic substituents of phenylalkylamines.

A second line of work in connection with the derivatives of glyoxaline has been followed in attempts to prepare bases of pilocarpine-like action. The results of such experiments, however, have been entirely negative and serve to show that like other groups of compounds, such as the tropeines, the glyoxalines only become possessed of physiological activity under conditions difficult to determine.

Glyoxalines which are very closely related chemically vary enormously in physiological action, and a good example of this

is seen in the reduced activity of *iso*pilocarpine as compared with its stereo-isomeride pilocarpine.

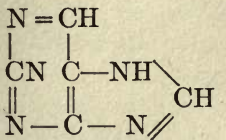
The pharmacology of the *iso*quinoline derivatives, a section which comprises a large number of well-known alkaloids, is, of course, a large subject, and we will consequently limit ourselves to a discussion of the relation between Chemical Constitution and Physiological Action in bases of the cotarnine type, of which there are now a number of known examples. The conclusion to which we are led in this case is that the property of causing contraction of the uterus is common to those 2-alkyl-3:4-dihydro-*iso*quinolinium bases (that is bases of the cotarnine type) which contain methoxy- or methylenedioxy-groups. At the same time we desire to qualify this statement by pointing out that only a comparatively small number of such compounds have been prepared and physiologically examined. Experience teaches that an apparent relation between Chemical Constitution and Physiological Action often appears to exist between a small number of closely allied substances, but on extending the field of enquiry somewhat wider, it is frequently found that no satisfactory generalisation can be deduced.

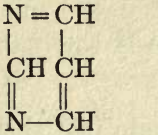
The third section of this paper deals with recent work on the relation between Chemical Constitution and Hypnotic Action, and for this purpose only those compounds containing the acid amide radicle, - CO - NH -, have been described. Under this heading such well-known groups as barbituric acid, urethane and their allied compounds can be included, and it was found advisable to limit the paper in this manner on account of the wide range of the subject.

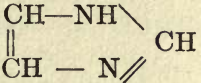
Glyoxaline Derivatives

The organic bases which occur in nature contain nitrogen combined in many different ways. Of the ring compounds with one nitrogen atom mention may be made of the pyridine, pyrollidine, quinoline, *iso*quinoline and indole derivatives. Rings containing more than one N-atom also occur, thus a large and important class of naturally occurring substances—the purine derivatives—

contain a double ring system, each containing two nitrogen atoms, the two rings being the pyrimidine and glyoxaline rings.

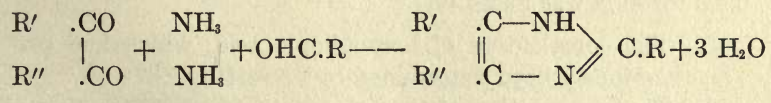

Purine


Pyrimidine


Glyoxaline

Until the last decade, however, the simple glyoxaline ring had not been recognised as a constituent of physiologically important compounds, although glyoxaline itself and some of its simpler derivatives had long been known. The first recognition of the simple glyoxaline ring in a naturally occurring compound was made in the case of the alkaloid *iso*pilocarpine; shortly afterwards the important amino-acid histidine was shown to be a glyoxaline derivative, and quite recently 4(or 5) β-aminoethylglyoxaline and ergothioneine, another glyoxaline derivative, have been isolated from ergot.

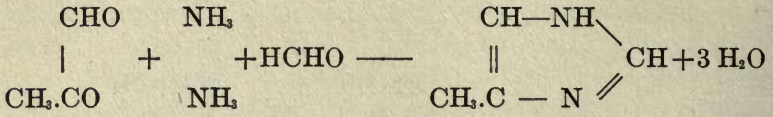
The occurrence of glyoxaline derivatives amongst natural products is susceptible of a ready explanation. Glyoxalines it is well-known are readily formed when ammonia is added to a cold aqueous solution containing an aldehyde, R.CHO, and a compound R′.CO.CO.R″, where R, R′, and R″ may be hydrogen as in the case of glyoxal itself



and many alkylglyoxalines have been prepared in the laboratory in this way. Moreover, it has been shown[1] that when an aqueous solution of glucose, formaldehyde and zinc hydroxide-ammonia is left exposed to light for some time 4(or 5)-methylglyoxaline is produced, and it seems probable that in this reaction the glucose

[1]Windaus and Knoop (*Ber.*, 1905, **38**, 1166).

is first degraded to methylglyoxal, which then enters into combination with the ammonia and formaldehyde as depicted below:

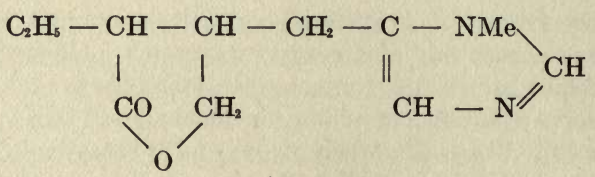


It is interesting to note that this reaction takes place under normal conditions of temperature and pressure, and requires only such reagents whose formation in nature can readily be understood.

Before going on to describe the synthetic experiments made with the view of preparing substances of physiological activity, we propose to give a short account of the recent researches on the naturally occurring glyoxaline derivatives.

Pilocarpine.

Pinner and Schwarz[1] first suggested that pilocarpine was a glyoxaline derivative, and proposed the constitutional formula (I)



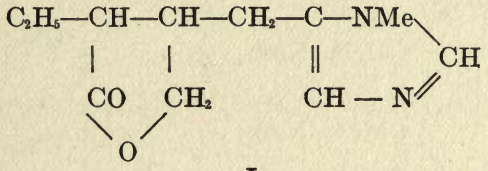
(I)

This formula was based on

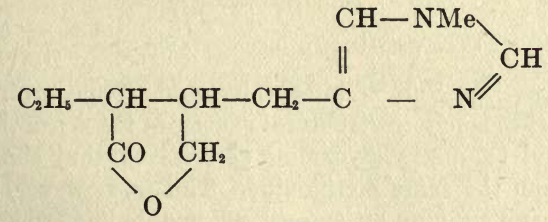
1. The constitution of homopilopic acid, which had previously been determined by Jowett[2].
2. The composition of the fragment left on subtraction of the homopilopic residue from the empirical formula of pilocarpine.
3. The formation of methylurea on oxidation, and
4. Certain analogies shown to exist between pilocarpine derivatives and glyoxalines.

[1] *Ber.*, 1902, **35**, 2441.
[2] *J. C. S. Trans.*, 1901, **79**, 1331.

Definite proof that pilocarpine is a glyoxaline derivative was furnished by Jowett[1] in the next year. By distilling *iso*pilocarpine with soda lime he isolated and identified 1-methylglyoxaline, 1:4(or 1:5)-dimethylglyoxaline and 1:4(or 1:5)-methylmylglyoxaline. For the purpose of comparison, Jowett and Potter[2] prepared what they believed to be a homogeneous 1:4(or 1:5)-dimethylglyoxaline by methylating 4(or 5)-methylglyoxaline, but came to the conclusion that this was not identical but isomeric with the dimethylglyoxaline from *iso*pilocarpine. As there was no evidence to show which of the two dimethylglyoxalines was the 1:4 isomeride and which the 1:5, Jowett put forward for *iso*pilocarpine the two alternative formulae (I) and (II) of which (I) is identical with that suggested by Pinner and Schwarz, and represented pilocarpine as a stereo-isomeride.



I



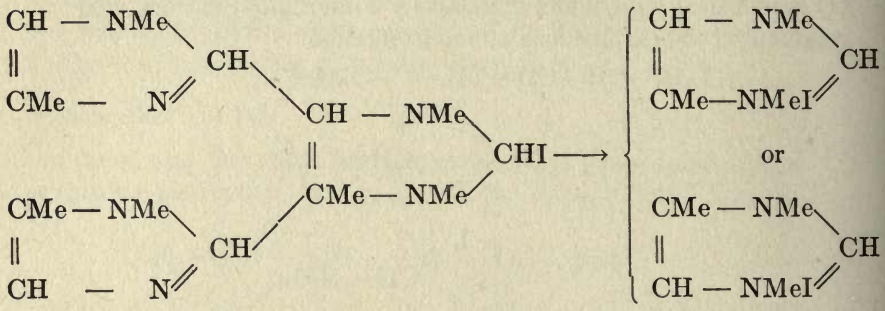
II

Pinner[3] regarded pilocarpine and *iso*pilocarpine as structural isomerides—derivatives of the two 1:4 and 1:5-methylglyoxalines corresponding with the formulae (I) and (II), but Jowett[4] was able to show that the alkaloids are not structural but stereo-isomerides, since they are mutually interconvertible by means of alcoholic potash.

[1] *J. C. S. Trans.*, 1903, **83**, 438.
[2] *J. C. S. Trans.*, 1903, **83**, 464.
[3] *Ber.*, 1905, **38**, 1510.
[4] *J. C. S. Trans.*, 1905, **87**, 794.

Recently Pyman[1] has repeated the methylation of 4(or 5)-methylglyoxaline, and isolated the two isomerides 1:4 and 1:5-dimethylglyoxaline. The latter proved to be identical with the dimethylglyoxaline obtained by Jowett by the distillation of *iso*pilocarpine with soda lime. *Iso*pilocarpine has therefore the formula (I).

Further evidence was also obtained against the view that the difference between pilocarpine and *iso*pilocarpine depends on structural isomerism as represented by Pinner. Thus, it was found that 1:4 and 1:5-dimethylglyoxaline yield one and the same methiodide, doubtless owing to tautomeric changes in the sense of the following scheme:



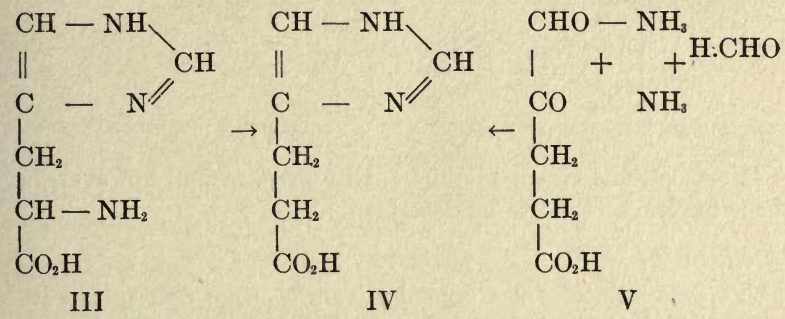
By analogy therefore if pilocarpine and *iso*pilocarpine owed their isomerism solely to structural causes, in the sense that they were 1:4 and 1:5-methylglyoxaline derivatives, they should also yield one and the same methiodide. This is, however, not the case, pilocarpine giving an amorphous methiodide, whereas *iso*-pilocarpine methiodide is crystalline. These facts, therefore, afford further evidence against Pinner's view of the isomerism of pilocarpine and *iso*pilocarpine, and consequently strengthen Jowett's position.

Histidine.

Histidine is an amino-acid which occurs as a degradation product of most albumins. It is readily prepared by hydrolysing

[1] *J. C. S. Trans.*, 1910, **97**, 1814.

haemoglobin. Pauly[1] first suggested the constitution of histidine (III) which is now known to be correct, but it was Knoop and Windaus[2] who first proved that this amino-acid is a glyoxaline derivative by degrading it to β-glyoxaline-4(or 5)-propionic acid (IV) which they also prepared synthetically by the action of formaldehyde and ammonia on glyoxylpropionic acid (V).



III IV V

The recent synthesis of histidine by Pyman will be referred to later.

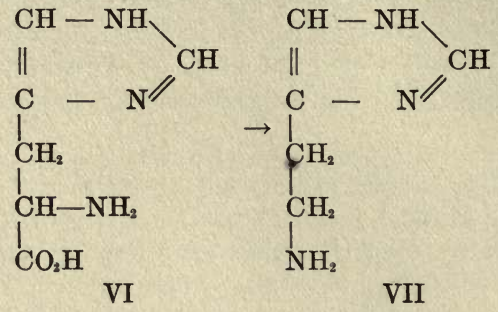
4(or 5)-β-Aminoethylglyoxaline.

This base was first prepared synthetically[3] by the degradation of β-glyoxaline-4(or 5)-propionic acid (IV) by Curtius' method some years before it was recognised as a naturally-occurring compound. Recently, however, it has been shown to be present in certain preparations of ergot,[4] and to have very great physiological activity.[5]

4(or 5)-β-Aminoethylglyoxaline (VII) can be prepared from histidine (VI) in the laboratory by the elimination of carbon dioxide by bacterial action,[6] and it seems reasonable to suppose that it is produced in this manner in nature. It can also be

[1] *Zeitsch. physiol. Chem.*, 1904, **42**, 513.
[2] *Beitr. chem. Physiol. Path.*, 1905, **7**, 144.
[3] Windaus and Vogt (*Ber.*, 1907, **40**, 3691).
[4] Barger and Dale (*J. C. S. Trans.*, 1910, **97**, 2592).
[5] Dale and Laidlaw (*J. physiol.* 1910, **41**, 318).
[6] Ackermann (*Zeitsch. physiol. Chem.*, 1910, **65**, 504).

prepared from histidine by removing carbon dioxide by purely chemical means, such as the use of acids at a high temperature.[1]

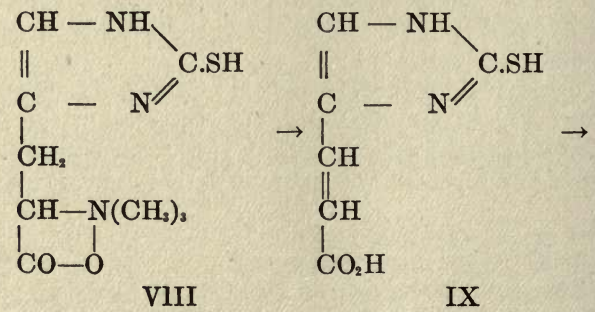


VI VII

The most convenient method for its preparation, however, is the synthetic method to be described.

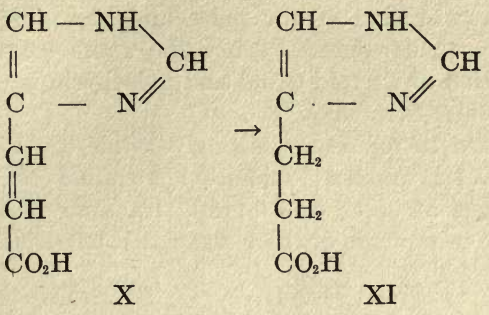
Ergothioneine.

This was isolated from ergot by Tanret[2] who determined its composition, $C_9H_{15}O_2N_3S$. Barger and Ewins[3] have recently shown that this compound is β-2-thiolglyoxaline-4(or 5)-propiobetaine (VIII) in the following manner. On boiling the base with strong aqueous potassium hydroxide, trimethylamine was removed, and β-2-thiolglyoxaline-4(or 5)-acrylic acid (IX) resulted; on oxidation with nitric acid this gave β-glyoxaline-4 (or 5)-acrylic acid (X) and the latter compound furnished β-glyoxaline-4(or 5)-propionic acid (XI) on reduction; the last two acids mentioned were identical with snythetically prepared specimens.



VIII IX

[1]Ewins and Pyman (*J. C. S. Trans.*, 1911, **99**, 339).
[2]*J. Pharm. Chim.* 1909 (VI), **30**, 145.
[3]*J. C. S. Trans.*, 1911, **101**, 2336.



X XI

Ergothioneine has no marked physiological action. It is an interesting addition to the comparatively small number of plant principles containing sulphur, and is the first example of a 2-thiolglyoxaline to be found in nature.

Iodated Proteins.

It will be observed that apart from pilocarpine and the allied alkaloids the glyoxaline derivatives which have hitherto been isolated from natural sources are derivatives of or nearly related to histidine. The latter is widely distributed in nature in combination with other amino-acids, entering into the composition of most albumins, and it has recently been suggested that naturally-occurring iodated proteins may contain the iodine fixed in the glyoxaline nucleus of histidine residues.

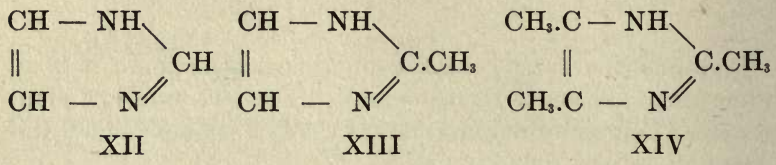
The active principle of thyroid glands, for instance, has been shown[1] to be an iodated globulin, and in view of its physiological importance, the nature of the iodine-bearing group in this compound, iodothyrin, has been the subject of investigations by Pauly and Gundermann.[2] These authors reviewed the various amino-acids which are formed by the hydrolysis of proteins, and showed that histidine is the one which can most readily fix iodine permanently when treated with gentle iodating agents, such as iodine and alkali. They therefore prepared and tested a number of iodated and brominated glyoxalines, and found that the halogenated glyoxalines (unlike the halogen free bases) caused

[1] Baumann (*Zeitschr. physiol. Chem.* 1895, **21**, 319).

[2] *Ber.*, 1908, **41**, 3999; 1910, **43**, 3243 and *Arch. expt. Path. Pharm.* 1911, **65**, 259.

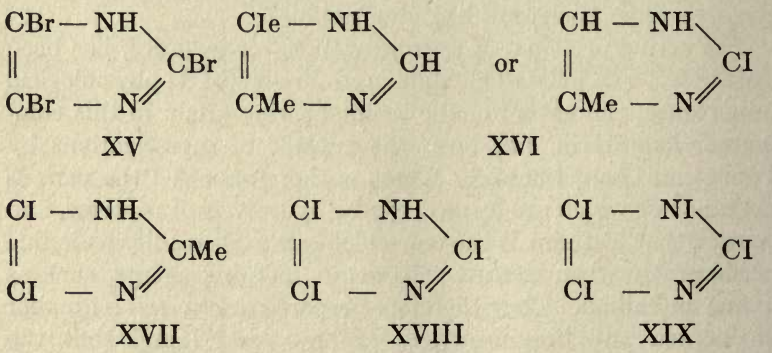
a marked acceleration of the pulse and breathing frequency; this is of particular interest since the characteristic pharmacological action of thyroid gland and iodothyrin is the acceleration of the pulse-frequency.

With regard to toxicity, whilst glyoxaline (XII), 2-methylglyoxaline (XIII) and 2:4:5-trimethylglyoxaline (XIV) had little effect on dogs in doses of 1.0-gram, fractions of this amount proved toxic in the case of the halogenated bases.



XII XIII XIV

The most toxic compound tested was 2:4:5-tribromoglyoxaline (XV) of which 0.2-gram given per os was sufficient to kill a 6½-kilo dog in 2 hours. Of the iodated glyoxalines 2(or 5)-iodo-4-methylglyoxaline (XVI) was the most toxic 0.3-grams per os killing a medium weight dog in 10 hours, then followed 4:5-diiodo-2-methylglyoxaline (XVII) of which the lethal dose was 0.4-grams and 2:4:5-triiodoglyoxaline (XVIII) of which the lethal dose was 0.6-grams or more.



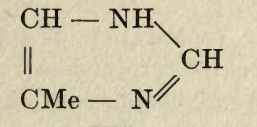
XV XVI

XVII XVIII XIX

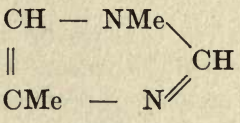
1:2:4:5-tetraiodoglyoxaline (XIX) and tetraiodohistidineanhydride had little or no pharmacological action probably owing to their slight absorption from the intestine.

Synthetic Glyoxaline Derivatives and Their Physiological Action.

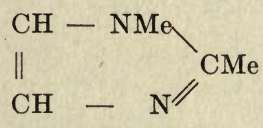
Until quite recently synthesis in the glyoxaline series has been confined almost exclusively to the preparation of glyoxalines with simple substituents such as alkyl-groups. A few of these appear to have been physiologically tested; thus 4(or 5)-methylglyoxaline (XX) is described as toxic.[1] Further, 1:4-dimethylglyoxaline (XXI) and 1:2-dimethylglyoxaline (XXII) are said to have no pilocarpine-like action,[2] whilst 1-ethyl-2-methylglyoxaline (XXIII) (oxalethylin) is stated to show a surprising similarity in its action to that of atropine.[3]



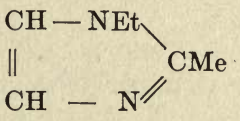
XX



XXI



XXII



XXIII

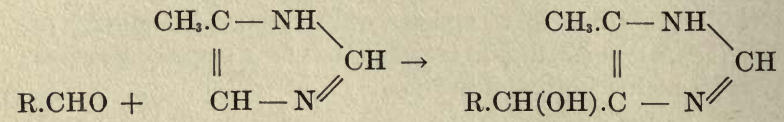
and apart from the fact that considerably larger doses are necessary to cause all the characteristic effects of atropine. In view of this statement we have prepared a quantity of 1-ethyl-2-methylglyoxaline by Radziszewski's method[4] and Dr. H. H. Dale who has tested it finds that 4-drops of 4% solution instilled into the eye of a cat produced no trace of mydriatic action; 100-mgms. did not paralyse the heart vagus or the action of the chorda tympani on salivary secretion, and the only trace of atropine-like action exhibited was seen in its antagonistic action to that of pilocarpine when directly applied to the frog's heart.

Within the last few years, however, glyoxalines with longer side chains have been synthesised; Knoop and Windaus' synthe-

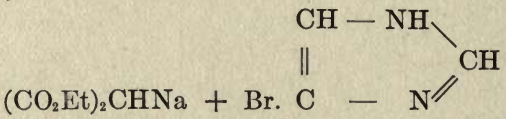
[1]Kowalewski (*Biochem. Zeitschr.*, 1909, **23**, 1).
[2]Jowett (*J. C. S. Trans.*, 1903, **83**, 466; 1905, **87**, 406).
[3]Schulz (*Ber.*, 1880, **13**, 2353).
[4]*Ber.*, 1883, **16**, 489.

sis (in 1905) of β-glyoxaline-4(or 5)-propionic acid from glyoxylpropionic acid and their degradation of the former compound to 4(or 5)-β-aminoethylglyoxaline have already been mentioned.

Further the discovery[1] that 4(or 5)-methylgloyxaline can be readily prepared in quantity from glucose has led to a number of interesting researches, [2], [3], [4] in which the base has been condensed with variou aldehydes to yield alcohols in accordance with the following scheme:



In 1905 after clearing up the constitution of pilocarpine, Jowett[5] suggested the preparation of pilocarpine-like compounds by the condensation of brominated glyoxalines with substances such as ethyl sodiomalonate.



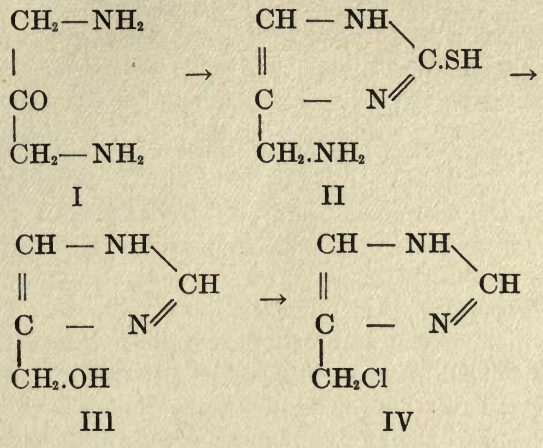
but at the time brominated glyoxalines were not readily accessible and no actual condensation experiments were carried out.

Some years afterwards Pyman[6] prepared quantities of several glyoxalines brominated in the ring, but found that they would neither react with compounds of the type of ethylsodiomalonate, nor would they react with magnesium to form glyoxaline magnesium bromides, and were therefore useless for synthetic purposes. Later, however, the same author[7] devised a method by which 4(or 5)-chloromethylglyoxaline could readily be prepared.

Diaminoacetone dihydrochloride (I) was condensed with one molecule of potassium thiocyanate and the resulting product

[1]Windaus and Knoop (*Ber.*, 1905, **38**, 1166).
[2]Gerngross (*Ber.*, 1909, **42**, 398; 1912, **45**, 509).
[3]Windaus (*Ber.*, 1909, **42**, 758).
[4]Ewins (*J. C. S. Trans.*, 1911, **99**, 2052).
[5]*J. C. S. Trans.*, 1905, **87**, 405.
[6]*J. C. S. Trans.*, 1910, **97**, 1814; 1912, **101**, 530.
[7]*J. C. S. Trans.*, 1911, **99**, 668.

(II) treated with nitric acid yielding 4(or 5)-hydroxymethylglyoxaline (III); this on treatment with phosphorous pentachloride gave 4(or 5)-chloromethylglyoxaline (IV).



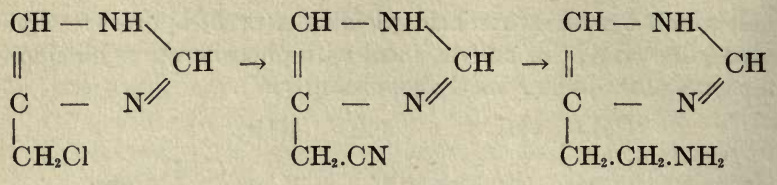
The latter compound contains the chlorine atom in a very reactive condition and may be employed for the introduction of the glyoxaline methyl ($C_3H_3N_2.CH_2$) group into organic compounds in the same way that benzyl chloride is used for the introduction of the benzyl group.

Mainly by the use of this compound it has been possible to carry out the following researches:

1. Synthesis and physiological examination of various aminoalkylglyoxalines.
2. Synthesis of histidine.
3. Synthesis and physiological examination of various glyoxaline derivatives containing carboxylic groups.

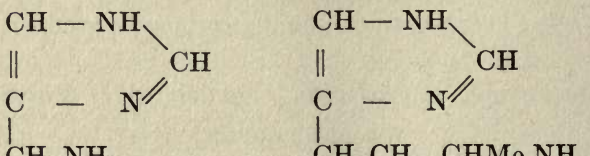
1. *Synthesis and Physiological Examination of Various Aminoalkylglyoxalines.*

In the first instance, 4(or 5)-β-aminoethylglyoxaline (VI) was synthesised from this compound by replacing the chloro-group by the cyano-group forming 4(or 5)-cyanomethylglyoxaline (V), which was then suitably reduced.



IV V VI

Physiological examination of these compounds showed that Nos. (II), (III), (IV) and (V) were almost devoid of stimulant action on the uterus, and had only trivial pressor effects on the blood pressure, whilst 4(or 5)-β-aminoethylglyoxaline (VI) has a very powerful motor effect on the isolated uterus, and a well marked depressor effect upon the blood pressure. The preparation of homologous aminoalkylglyoxalines might therefore be expected to lead to interesting results. Barger and Dale[1] in dealing with the relationship between the chemical constitution of the amines and their physiological action, have shown that the activity varies greatly with the length of the side-chain; in the fatty series the maximum of activity is attained at hexylamine, whilst the most active phenylalkylamine is phenylethylamine, having a fatty side-chain of two carbon atoms. It appeared, therefore, of interest to determine the optimum length of side-chain for physiological effect in the aminoalkylglyoxalines.[2] For comparison with 4(or 5)-β-aminoethylglyoxaline, 4(or 5)-aminomethylglyoxaline (VII) and 4(or 5)-γ-aminopropylglyoxaline were required, but since the latter was not readily accessible its methylhomologue, 4(or 5)-γ-aminobutylglyoxaline (VIII) was prepared and tested in its place.



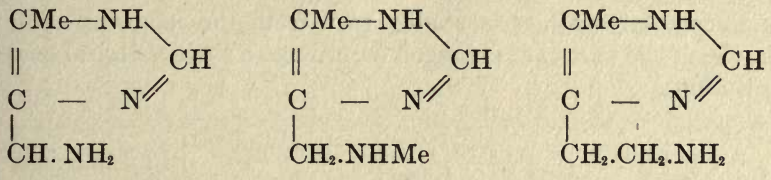
VII VIII

[1] *J. Physiol.*, 1910, **41**, 19.

[2] Pyman (*J. C. S. Trans.*, 1911, **99**, 2172).

Neither of these bases had any physiological action at all comparable with that of No. (VI), both of them only producing very weak motor effects on the uterus, and very faint pressor effects on the blood pressure.

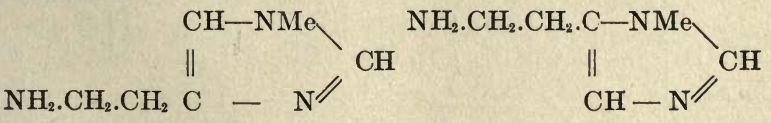
A similar relation obtains in the homologous series derived from 4(or 5)-methylglyoxaline.[1] Here again the aminomeyethyl derivatives, 4(or 5)-methyl-5(or 4)-aminomethylglyoxaline (IX) and 4(or 5)-methyl-5(or 4)-methylaminomethylglyoxaline (X) are physiologically almost inactive, whilst the ethylamine derivative (XI) has considerable physiological activity.



IX X XI

This base, 4(or 5)-methyl-5(or 4)-β-aminoethylglyoxaline produced a fall in blood pressure similar to, though somewhat less powerful than, that of 4(or 5)-β-aminoethylglyoxaline when injected intravenously. Its motor effect on plain muscle however, was far less than that of the last mentioned base.

The next point investigated was the effect of substituting the imino-hydrogen atom in (VI) by the methyl-group. The two isomeric *N*-methyl derivatives 1-methyl-4-β-aminoethylglyoxaline (XII) and 1-methyl-5-β-aminoethylglyoxaline (XIII) were accordingly prepared,



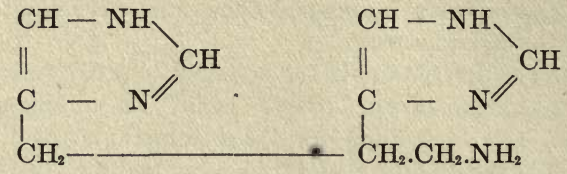
XII XIII

and tested. Their physiological action, however, was negligible compared with that of the parent compound (VI).

β-γ-Bis[4(or 5)-glyoxaline] propylamine (XIV) which may be regarded as an aminoethylglyoxaline containing a glyoxa-

[1]Ewins (*J. C. S. Trans.*, 1911, **99**, 2054).

linemethyl substituent was also tested physiologically; the effect of this base on the uterus and blood pressure is barely perceptible.



XIV

The nature of the results obtained by the investigation of these aminoalkylglyoxalines is readily seen from the following table in which the bases are arranged according to their chemical constitution:

Ref.No.	Base	Physiological Activity
(VII)	$C_3H_3N_2.CH_2.NH_2$	Slight
(IX)	4:5-Me. $C_3H_3N_2.CH_2.NH_2$	Slight
(X)	4:5-Me. $C_3H_3N_2.CH_2.NHMe$	Slight
(VI)	$C_3H_3N_2.CH_2.CH_3.NH_2$	Very great
(XI)	4:5-Me. $C_3H_3N_2.CH_2.CH_2.NH_2$	Considerable
(XII)	1:4-Me. $C_3H_3N_2.CH_2.CH_2.NH_2$	Slight
(XIII)	1:5-Me. $C_3H_3N_2.CH_2.CH_2.NH_2$	Slight
(XIV)	$C_3H_3N_2.CH_2$	
	$C_3H_3N_2.CH.CH_2.NH_2$	Slight
(VIII)	$C_3H_3N_2.CH_2.CH_2.CHMe.NH_2$	Slight

Amongst the aminoalkylglyoxalines tested therefore only those two (VI) and (XI) have any pronounced physiological action in which

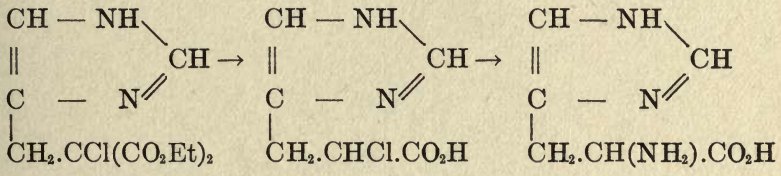
(1) the amino-group and the glyoxaline complex are connected by a chain of two carbon atoms, and

(2) the imino-group of the glyoxaline complex is free.

2. *Synthesis of Histidine.*

4(or 5)-chloromethylglyoxaline readily reacts with compounds of the type of ethyl sodiomalonate forming condensation products, and histidine has been synthesised by this means as follows:[1]

4(or 5)-chloromethylglyoxaline was condensed with ethyl sodiochloromalonate to give ethyl 4(or 5)-glyoxalinemethylchloromalonate (XV); this on hydrolysis with hydrochloric acid gave α-chloro-β-glyoxaline-4(or 5)-propionic acid (XVI) which when treated with strong ammonia under pressure gave racemic histidine (XVII); the latter was then resolved into its optically active components by fractional crystallisation of the acid tartrates.



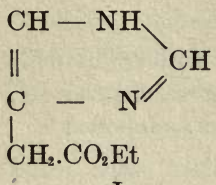
XV XVI XVII

3. *Synthesis and Physiological Examination of Various Glyoxaline Derivatives Containing Carboxylic Groups.*

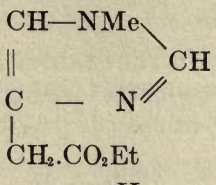
Starting with 4(or 5)-chloromethylglyoxaline it has been possible to synthesise a large number of compounds having this much in common with pilocarpine that they contain the glyoxaline complex, and an esterified carboxyl-group.[2] It may be stated at the outset that none of the compounds prepared had any pilocarpine-like action, and most of them were physiologically inactive.

In the first place a number of ethyl esters were prepared and tested. These were ethyl glyoxaline-4(or 5)-acetate (I), ethyl 1-methylglyoxaline-4-acetate (II), ethyl 4(or 5)-glyoxalinemethylmalonate (III), ethyl 4(or 5)-glyoxalinemethylmethylacetoacetate (IV), ethyl 4(or 5)-glyoxalinemethylchloromalonate (V) and 4(or 5)-glyoxalinemethylchloromalonamide (VI).

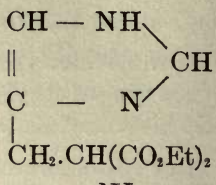
[1]Pyman (*J. C. S. Trans.*, 1911, **99**, 1386).
[2]Pyman (*loc. cit.*).



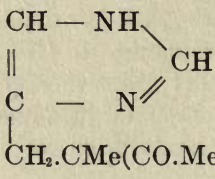
I



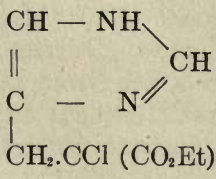
II



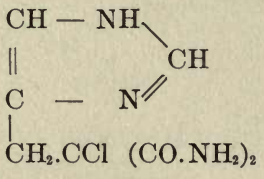
III



VI



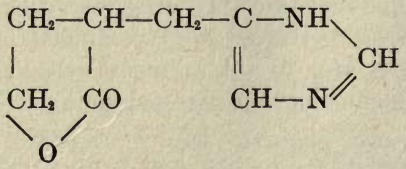
V



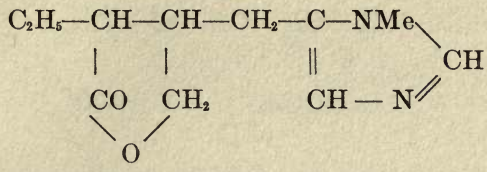
VI

The fact that none of these substances had any pilocarpine-like action showed that the presence of an esterified carboxyl-group in a glyoxaline derivative was not sufficient to confer the physiological properties characteristic of this alkaloid. It was thought, however, that if the carboxyl-group were internally esterified forming a lactonic complex as in pilocarpine, the desired effect might be produced. The lactone of ∝ (β-hydroyethyl)-β-glyoxaline-4(or 5)-propionic acid(VII) was therefore synthesised.

This compound has certain constitutional features in common with pilocarpine (VIII); thus both contain a glyoxaline residue connected through a methylene group with a butyryl lactone residue, though the point of attachment to the lactone ring

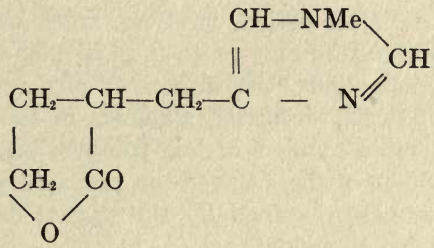


VII

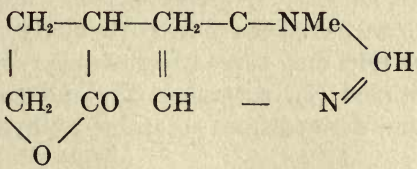


VIII

is different in the two cases. The two bases also differ in that the former is not methylated, and when it was found that the lactone (VII) was physiologically inactive, a quantity was methylated with a view to the preparation of the two isomeric N-methyl-derivatives (IX) and (X)



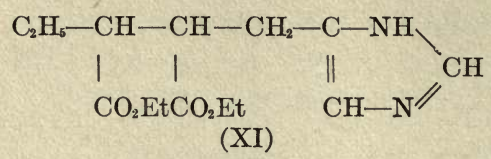
IX



X

Only one of these was isolated in a pure state, and this also was found to be physiologically inactive.

Another series of experiments, which cannot be described in detail here, was carried out with the object of synthesising pilocarpine, and resulted in the preparation of substances containing the skeleton of this alkaloid. Of these ethyl α-4(or 5)-glyoxalinemethyl-β-ethylsuccinate (XI) was submitted to physiological examination but likewise proved to be inactive.



(XI)

All attempts to synthesise glyoxaline derivatives of pilocarpine-like action have therefore hitherto been uniformly unsuccessful.

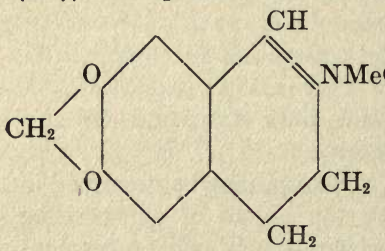
*ISO*QUINOLINE DERIVATIVES.

The constitution of many members of the large and important group of alkaloids containing the *iso*quinoline ring have been known for a long period, but it is only within the last few years that synthetic methods have been developed for their preparation. During the latter period, however, much work has been done in this field, and we may recall in particular the syntheses of laudanosine[1] papaverine,[2] berberine[3] and of narcotine.[4]

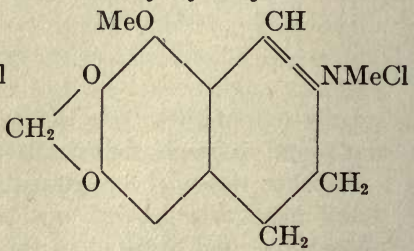
Besides the naturally-occurring alkaloids of the *iso*quinoline series, a considerable number of less complex bases have also been studied. Some of these have been prepared synthetically, whilst others are best obtained by the partial degradation of natural *iso*quinoline alkaloids.

We propose in this paper to deal with a special group of bases of the latter class, namely, the 2-alkyl-3:4-dihydro*iso*quinolinium bases with which we have been more particularly concerned.

Until the year 1909 only three bases of this type were known, namely, hydrastinine (I), cotarnine (II) and cotarnamic acid (III), a compound derived from cotarnine by hydrolysis.

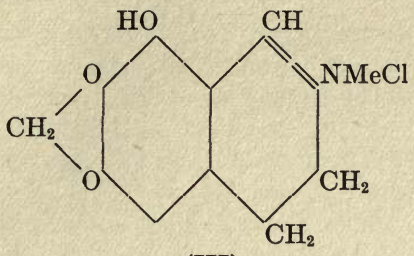


(I)



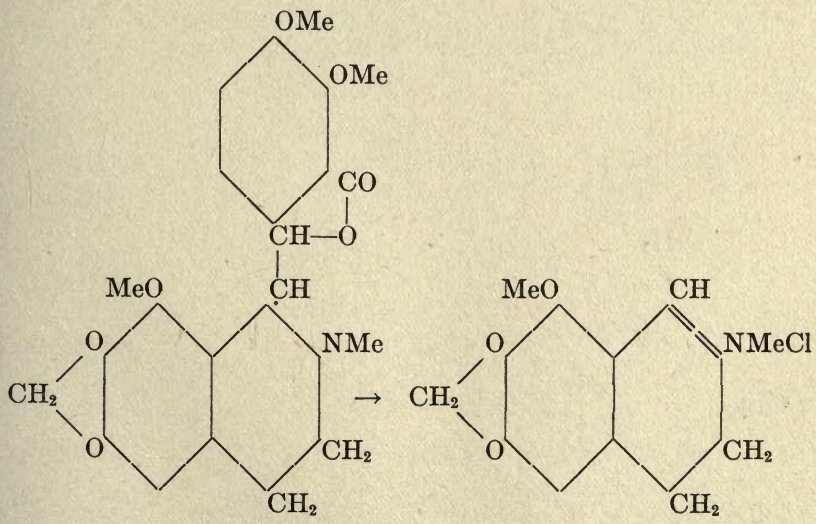
(II)

[1]Pictet & Finkelstein (*Ber.*, 1909, **42**, 1979).
[2]Pictet & Gams (*Ber.*, 1909, **42**, 2943).
[3]Pictet & Gams (*Ber.*, 1911, **44**, 2480).
[4]Perkin & Robinson (*J. C. S. Trans.*, 1911, **99**, 775).

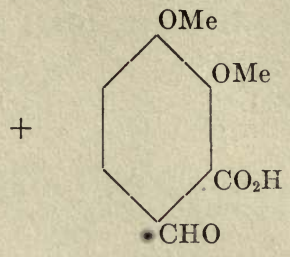


(III)

The only practical method at that time existing for the preparation of hydrastinine and cotarnine was the oxidative fission of hydrastine and narcotine (IV) respectively, when in each case opianic acid (VI) was obtained as a bye product. The following scheme depicts the oxidation of narcotine to cotarnine (V), and serves equally well to show the preparation of hydrastinine from hydrastine, when the methoxyl-groups in the 8-position of the *iso*quinoline rings are removed.

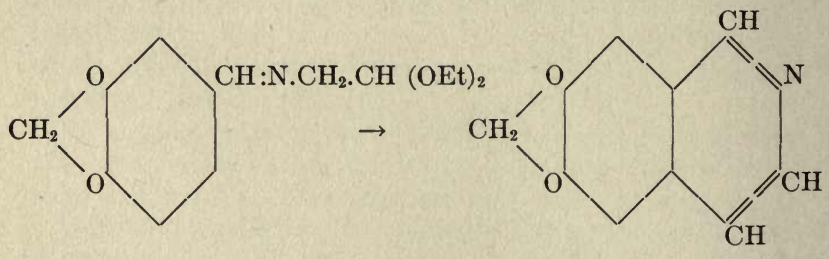


(IV) (V)

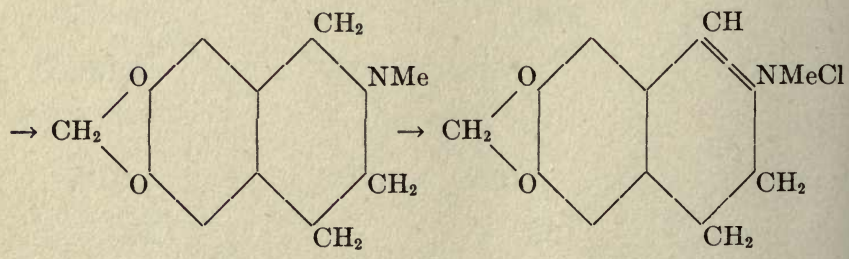


(VI)

No other method was known for the preparation of cotarnine, but hydrastinine had been synthesised in another way, which was not however, suitable for its preparation in quantity. This method[1] consisted in condensing 3:4-methylenedioxybenzylideneaminoacetal (VII) to 6:7-methylenedioxy*iso*quinoline (VIII) reducing a methyl salt of this base to hydrohydrastinine (IX) and oxidising the latter to hydrastinine (X)



(VII) (VIII)



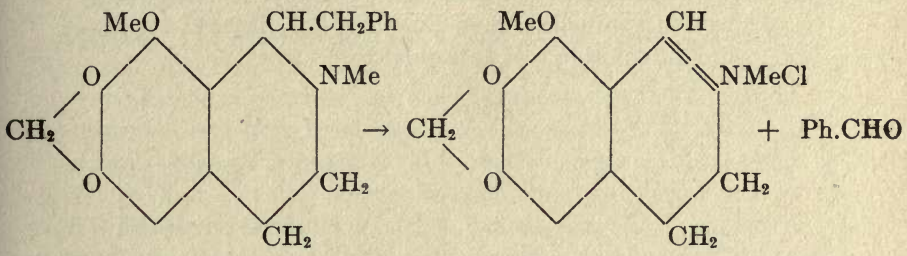
(IX) (X)

[5]Fritzsch (*Annalen*, 1895, **286**, 1)..

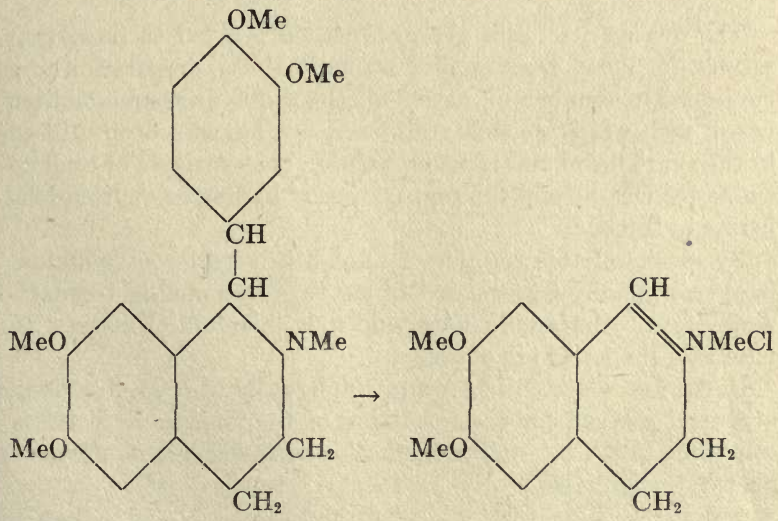
Since no other bases of the type of narcotine and hydrastine were known, cotarnine, hydrastinine and cotarnamic acid remained up till this time the only representatives of the 2-alkyl-3:4-dihydro*iso*quinolinium bases.

In 1909, however, Pyman[1] found that 1-benzyl-2-alkyltetrahydro*iso*quinolines in general gave on oxidation 2-alkyl-3:4-dihydro*iso*quinolinium bases together with the aldehyde corresponding with the substituted benzyl group: thus

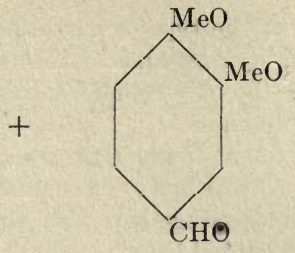
(1) 1-benzylhydrocotarnine gave cotarnine and benzaldehyde



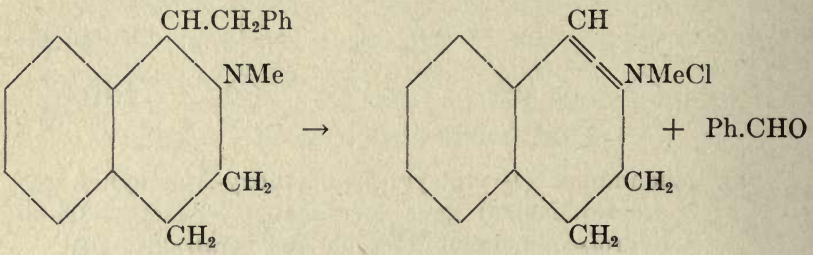
(2) laudanosine (1-veratryl-6:7-dimethoxy-2-methyltetrahydro*iso*quinoline) gave 6:7-dimethoxy-2-methyl-3:4-dihydro*iso*quinolinium chloride and veratraldehyde,



[1] *J. C. S. Trans.*, 1909, **95**, 1266, 1738.



(3) and in the simplest possible case, 1-benzyl-2-methyltetrahydro*iso*quinoline gave 2-methyl-3:4-dihydro*iso*quinolinium chloride and benzaldehyde.

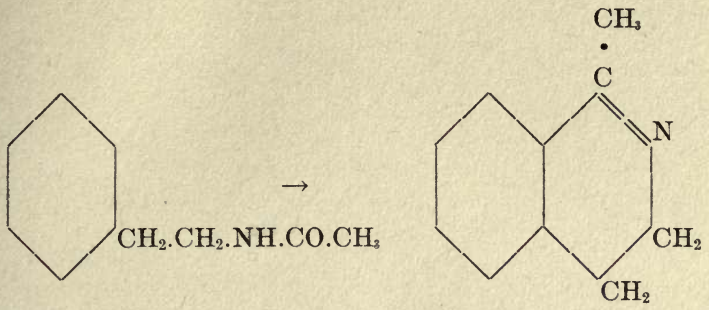


The discovery of this general method has led to important results, for it has been applied not only to the preparation of a considerable number of new 2-alkyl-3:4-dihydro*iso*quinolinium bases, with which we shall deal later, but has also been utilised in the synthesis of cotarnine by Salway, the synthesis of hydrastinine by Decker and the preparation of hydrastinine from berberine by Freund.

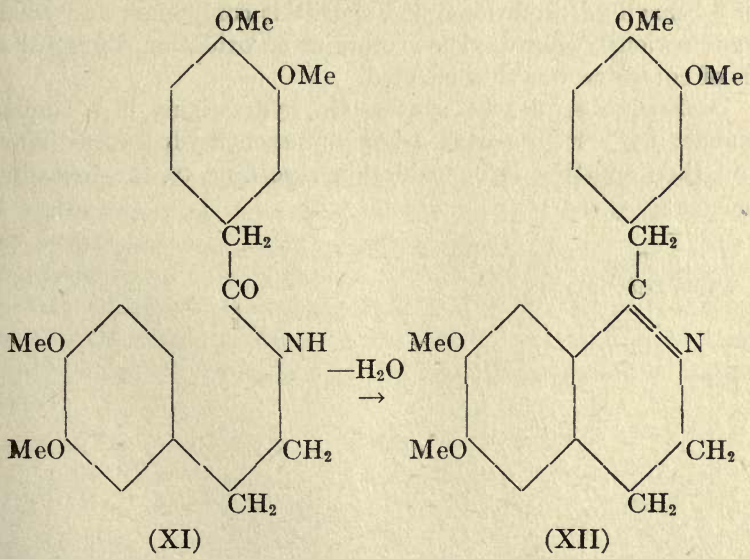
By means of this reaction, 2-alkyl-3:4-dihydro*iso*quinolinium bases can readily be prepared from the corresponding 1-benzyl-2-alkyltetrahydro*iso*quinolines, and it is therefore of interest to note how the latter are obtained.

In the first place, Bischler and Napieralski[1] in 1893, described a general method for the formation of *iso*quinoline derivatives consisting in the internal condensation of the acyl-derivatives of phenylethylamines.

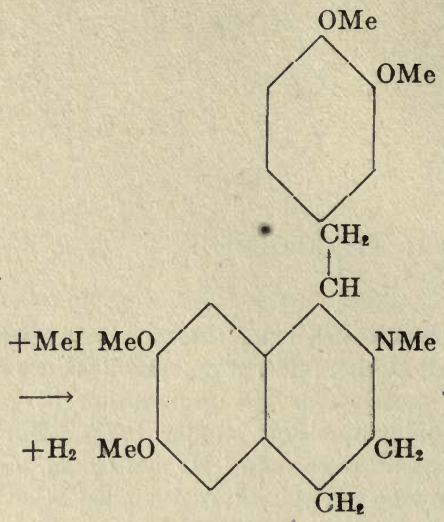
[1] *Ber.*, 1893, **26**, 1903.



Recently, the process has been thoroughly investigated by Pictet and Kay[1] and Decker and Kropp,[2] and has been developed into an excellent method for the preparation of 1-benzyl-2-alkyltetrahydro*iso*quinolines and similar bases. For instance, Picet and Finkelstein[3] were able to synthesise laudanosine (XIII) by first carrying out the internal condensation of homoveratroylhomoveratrylamine (XI) which they had prepared synthetically, then methylating the *iso*quinoline base (XII) so produced and reducing its methochloride.



[1] *Ber.*, 1909, **42**, 1973.
[2] *Ber.*, 1909, **42**, 2075.
[3] *Ber.*, 1909, **42**, 1979.



(XIII)

This method was applied by Salway[1] in 1910 to the synthesis of 1-benzylhydrocotarnine, and since this compound, as Pyman had previously shown, yields cotarnine on oxidation, the synthesis of cotarnine was thus effected.

Decker[2] subsequently synthesised hydrastinine in a similar manner by first preparing 1-homo-piperonylhydrohydrastinine, and then splitting it by oxidation according to the following scheme:

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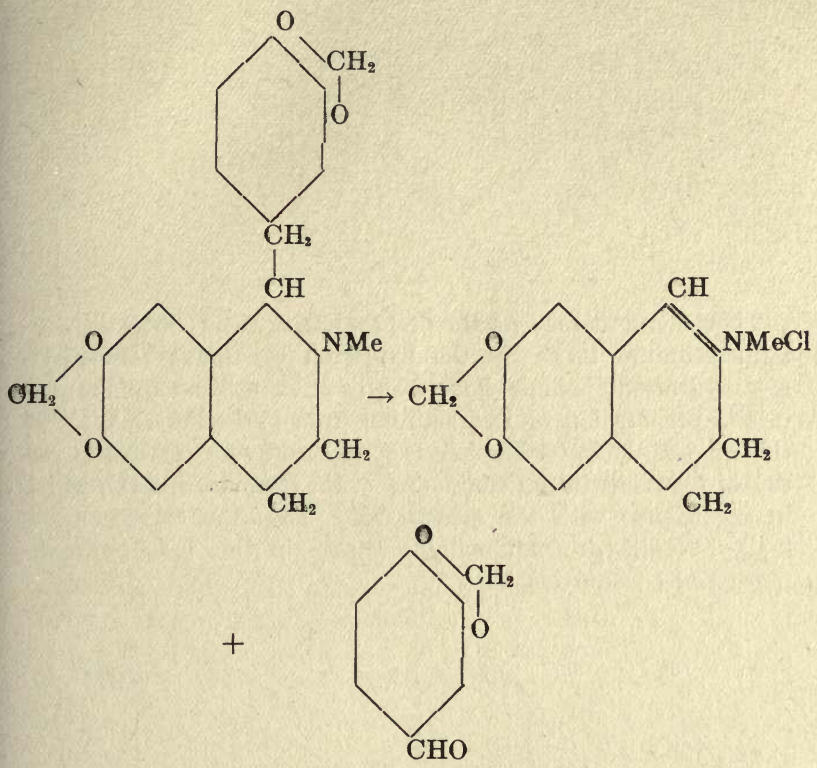
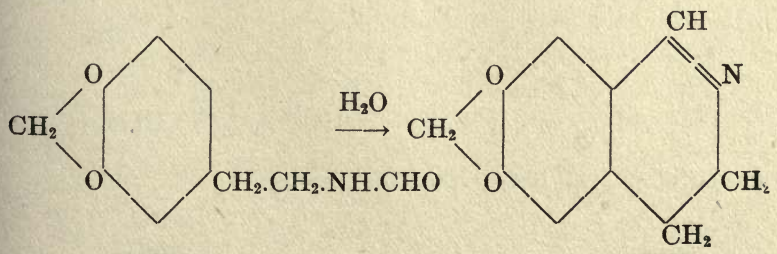
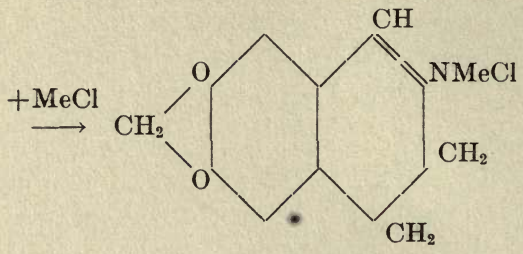
[1] *J. C. S. Trans.*, 1910, **97**, 1208.
[2] *Chem. Zeit.*, 1911, **35**, 1077.



The same author has also described a method for the syntheses of hydrastinine and cotarnine by the internal condensation of the formyl-derivatives of the corresponding phenylethylamine; thus formylhomopiperonylamine (XIV) gave rise to 6:7-methylenedioxy-3:4-dihydro*iso*quinoline (XV) of which the methochloride is hydrastinine chloride (XVI), but this method gave poor yields owing to the reaction proceeding mainly in another direction.

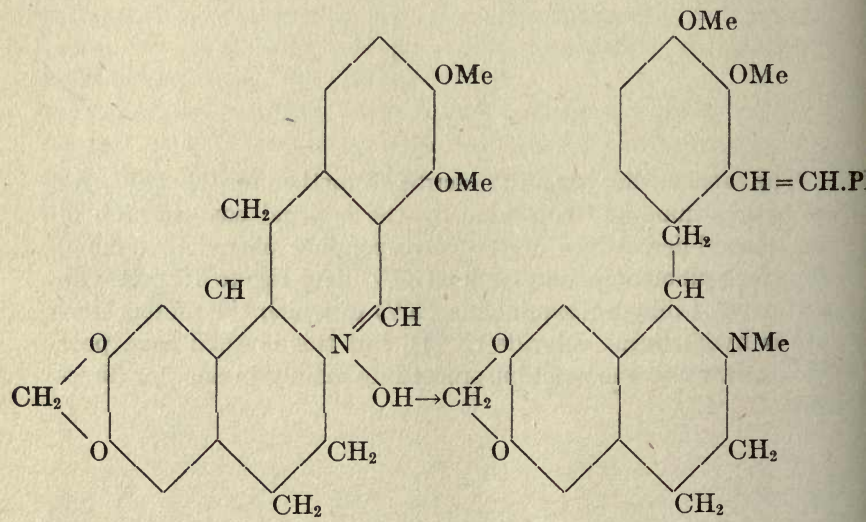


(XIV) (XV)



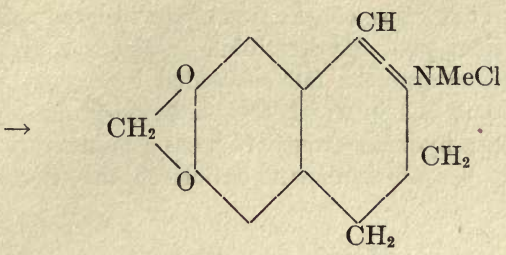
(XVI)

The general method for the preparation of 2-alkyl-3:4-dihydro-*iso*quinolinium bases by the oxidation of 1-benzyl-2-methyl-tetrahydro*iso*quinolines has recently been utilised by Freund[1] in the preparation of hydrastinine from berberine (XVII); in this process the latter alkaloid is converted by an interesting series of reactions into a compound of the formula(XVIII), which in accordance with the general rule yields the corresponding 2-alkyl-3:4-dihydro*iso*quinolinium base—in this case hydrastinine (XIX)—on oxidation.



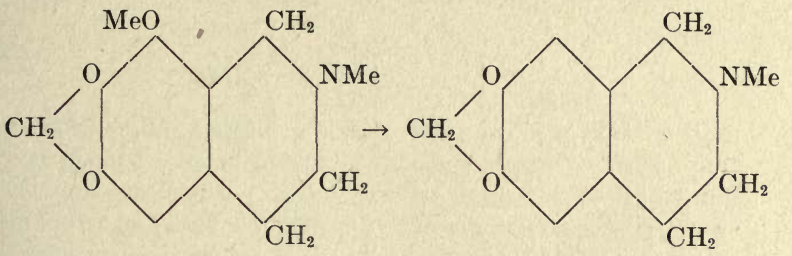
(XVII) (XVIII)

[1] *Chem. Zeit.* 1911, **35**, 1090.



(XIX)

Besides hydrastine and berberine, another naturally-occurring alkaloid, namely, narcotine may be used as a source of hydrastinine by means of the following process.[1] Narcotine is oxidised to cotarnine, and this reduced to hydrocotarnine (XX) by known methods; the latter base is then strongly reduced by means of sodium and alcohol, when the methoxyl-group is replaced by hydrogen and hydrohydrastinine (XXI) results.



(XX) (XXI)

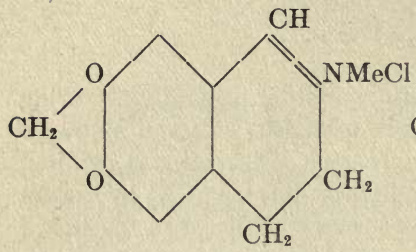
The latter base readily yields hydrastinine on oxidation.

Having now given some account of the methods applied to the synthesis of the previously known alkaloids cotarnine and hydrastinine, we propose to deal with a number of new 2-alkyl-3:4-dihydro*iso*quinolinium bases. All these have been prepared by the general method already described, that is the oxidation of the corresponding 1-benzyl-2-alkyl-tetrahydro*iso*quinoline.

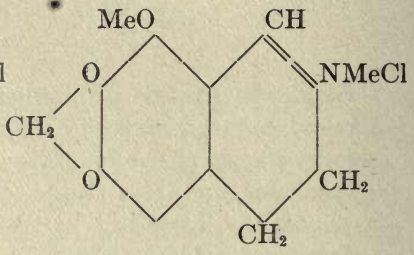
Hydrastinine (A) and cotarnine (B) have long been used in therapeutics as haemostatics particularly in abnormal uterin

[1]Wellcome, Pyman & Remfry (*Eng. Pat.* 23,736 of 1911).

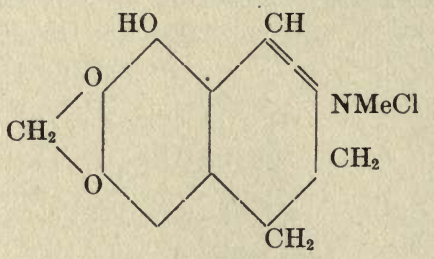
conditions. They cause contraction of the isolated uterus of cat, rabbit or guinea-pig. Hydrastinine occasions a rise of blood pressure, and cotarnine a fall succeeded by a very slight rise. Cotarnamic acid (C) in which the methoxyl-group of cotarnine is replaced by a hydroxy-group produces a minimal rise of blood pressure, but has no significant action on the uterus or other organ.



(A)



(B)



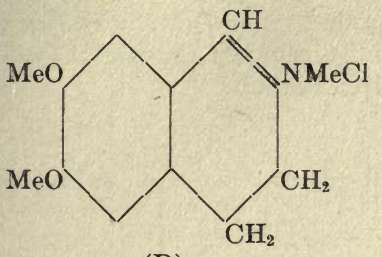
(C)

The new bases described below were prepared by one of us[1] except where otherwise stated, in an endeavour to produce an improved uterine haemostatic.

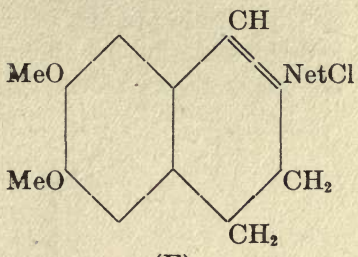
The physiological action of the most important of the new bases, 6:7-dimethoxy-2-methyl-3:4-dihydro*iso*quinolinium chloride (D) has already been fully described by Laidlaw[2] who has shown that this compound produces a well-marked contraction of the uterus, and a rise of blood pressure due to vaso-constriction and increased cardiac output; its action appears to be similar

[1]Pyman, (*J. C. S. Trans.*, 1909, **95**, 1266, 1738; 1910, **97**, 264).
[2]*Biochem., J.* 1910, **5**, 243.

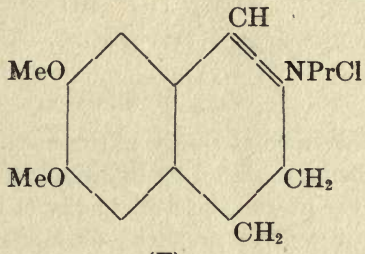
to that of hydrastinine. Clinical reports have shown that it is of great value in abnormal uterine conditions. It is slightly more toxic than cotarnine.



(D)



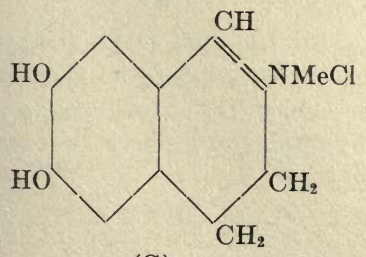
(E)



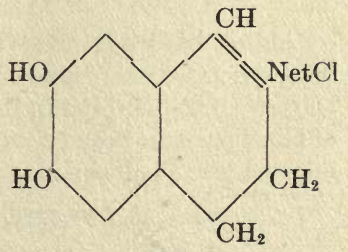
(F)

A considerable number of bases differing only slightly from (D) in chemical constitution has been prepared. In the first place, the compounds (E) and (F) in which the methyl-group on the nitrogen atom is replaced by the ethyl- and propyl-groups respectively were made; of these (E) proved to be very similar in its general action to (D), but was considerably more toxic. In its action on the blood pressure, (E) resembles cotarnine causing a fall, succeeded by a very slight rise.

Then, the corresponding dihydroxy-bases (G) and (H) were prepared from (D) and (E) respectively by hydrolysis:

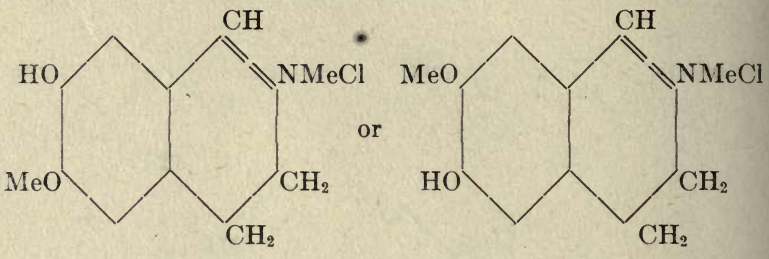


(G)



(H)

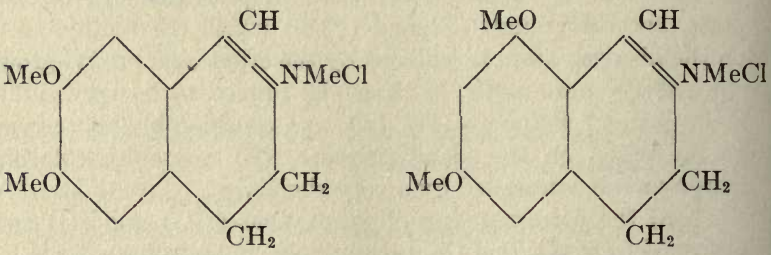
Of these, (G) was physiologically examined, but produced only a minimal rise of blood pressure, and had no significant action on the uterus, thus behaving like cotarnamic acid (C), the hydrolytic product of cotarnine. 6(or 7)-Methoxy-7(or 6)-hydroxy-2-methyl-3:4-dihydro*iso*quinolinium chloride (J) which has one of the two formulae given below:



(J)

represents an intermediate stage in the hydrolysis of (D) to (G), one methoxyl being replaced by hydroxyl; this compound causes contraction of the uterus and a slight rise of blood pressure.

Salway[1] has recently prepared 6:8-dimethoxy-2-methyl-3:4-dihydro*iso*quinolinium chloride (K), which is isomeric with (D), differing only from it in the position of one of the methoxy-groups as is shown below.

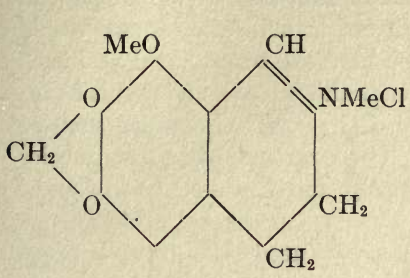


(D) (K)

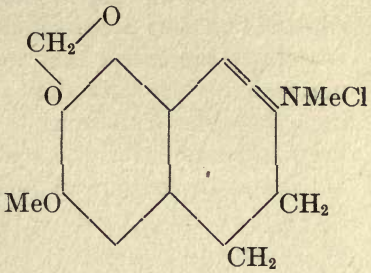
Laidlaw has shown that (K) closely resembles (D) in its action on the isolated uterus, and is considerably less toxic than either (D) or cotarnine. It further resembles (D) in producing a rise of blood pressure accompanied by slowing of the heart beat when injected into the blood stream of a cat.

[1] *J. C. S. Trans.*, 1911, **99**, 1320.

Salway[1] has also prepared *neo*cotarnine (L) an isomeride of cotarnine (C) having the relation to it shown below:



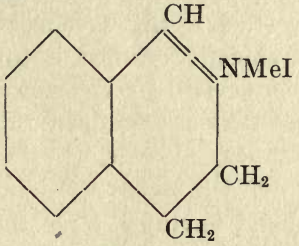
(C)



(L)

but unfortunately no account of its physiological action has yet been published.

The simplest possible example of this type of substance, namely, 2-methyl-3:4-dihydro*iso*quinolinium iodide (M) has also been prepared and tested.



(M)

Its action, however, is peculiar in that it produces in doses of 10 to 20 milligrams a marked rise of blood pressure superficially similar to that produced by adrenine.

It will be clear from a consideration of these results that the property of causing contraction of the uterus is common to all those 2-alkyl-3:4-dihydro*iso*quinolinium bases tested which contain only methoxy- or methylenedioxy-groups as substitutes; where these are replaced by hydroxy-groups (except in the case of J) this property appears to be lost or at least seriously diminished.

[1] *J. C. S. Trans.*, 1910, **97**, 1208.

Acid Amides

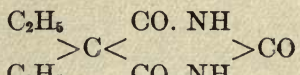
The title of this paper being the relationship between chemical constitution and physiological action in acid amides, we would explain at the outset that we use here the term " acid amides " in a rather wider sense than is usual and include under this heading substances containing the — CO — NH — group, such as urethanes and barbituric acids. The large majority of hypnotics are found to contain an NH_2 group and Fränkel[1] has brought forward evidence to show that even when several ethyl-groups are present in the molecule an unsubstituted NH_2 group is often necessary to impart to it hypnotic properties. Thus he regards ethyl urethane as an ester of an acid amide and not as an amino acid, for the reason that if an amino-acid, then the next higher gomologue, ethyl glycollate, should have hypnotic properties, but as a fact has none. In the light of this reasoning several large classes of hypnotic substances can fairly be brought within the scope of the present paper. With the theory of narcosis we are not specially concerned as that is more a question for the physiologist than the chemist. Suffice it to say that the principal one has been that devised by Overton and Meyer, which, put briefly, states that the more soluble a substance is in fats (lipoid substance) and the less in water, i.e., the greater the ratio of the solubility fat: water, the higher will be its hypnotic power.

Thus a compound having a large solubility ratio will be a stronger hypnotic than one having a smaller ratio.

Although giving results agreeing very closely with those found in practice, it has not held undisputed sway, and several new theories have been put forward of late years. For them we would refer readers to the original papers or to an excellent condensed exposition of the subject in Fränkel's Arzneimittelsynthese, 3rd edition 1912, page 510.

The trend of modern investigation has followed rather closely on those lines which have proved most successful in the past and the splendid results obtained by the use of diethylbarbituric acid (I)

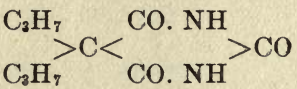
[1] *Arch. expt. Path. Pharm.* 1908, suppl. 181.



(I)

have led to the production of an enormous number of closely allied derivatives in the hope that an even safer and more effective hypnotic could be thus obtained.

Dipropylbarbituric acid (II) is the only derivative in which a change in the alkyl groups has effected an increase in hypnotic power. This, however, has been found to be almost too powerful in its action and consequently dangerous for general use.

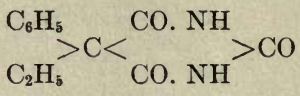


(II)

In order to obtain an hypnotic of the same order of activity as diethylbarbituric acid, but one of which it was necessary to take less in order to produce the same effects, the sodium derivative of this acid was introduced. It was expected that its much greater solubility in water would enable it to exert a prompter action and in this way have the same effect as a larger dose of the free acid. These expectations, however, were not realised in practice, for very little real difference was found in the rapidity with which sleep was induced by the two compounds.

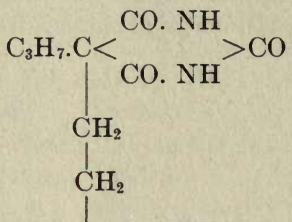
In the light of the Overton-Meyer theory this is not to be wondered at as the sodium derivative is probably quite insoluble in lipoid substances and hence, before action can take place, has to be decomposed and the free diethylbarbituric acid liberated by the acids of the body.

Dibenzylbarbituric acid has already been proved inactive, but just as acetamide (which also has no hypnotic effect) is endowed with slight hypnotic properties by the introduction of a phenyl radicle as in phenylacetamide, $C_6H_5.CH_2.CO.NH_2$, so inactive ethylbarbituric acid is said to be converted to a safe and active hypnotic when transformed into the phenyl-derivative (III).



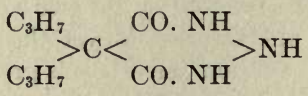
(III)

The joining together of two molecules of propylbarbituric acid by an ethylene linkage as in ethylene bis-5-propylbarbituric acid[1] (IV) resulted in an inactive compound although it may be regarded as ethylpropylbarbituric acid substituted on the β carbon atom of the ethyl radicle by a molecule of propylbarbituric acid.



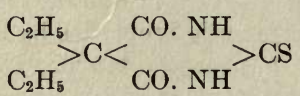
(IV)

Any attempt, however, to interfere with the barbituric acid ring dooms the product to failure as far as useful hypnotic properties go, for example, dipropylmalonylguanidine (V) is inactive.



(V)

Diethyl-*N*-methylbarbituric acid is very poisonous although still a strong hypnotic and the same occurs in the case of diethylmalonylthiourea (VI).



(VI)

[1]Remfry (*Trans.*, 1911, 99, 623).

The poisonous properties imparted by the methylation of the imino-group are analogous to the case of methyl benzamide (C_6H_5. CO. NH. CH_3) which has a strychnine-like action whilst benzamide itself has a slight alcohol-like narcotic effect. Similarly *N*-methylphenacetin is much more poisonous than phenacetin.

Einhorn[1] has described a great number of what may be regarded as derivatives of diethylmalonamide, in which the carbon atom joining the two nitrogen atoms has had different groups attached to it, such as

$$\begin{matrix} C_2H_5 \\ C_2H_5 \end{matrix}\!\!>\!C\!<\!\!\begin{matrix} CO.\,NH \\ CO.\,NH \end{matrix}\!\!>\!C\!=\!N.\,C_6H_5$$

These, however, all proved to be inactive.

Other examples of substances which differ only slightly in the construction of the ring, but still contain at least two ethyl or propyl radicles attached to one carbon atom and are yet inactive, are given in the following:

Diethylketopiperazine[2]

$$\begin{matrix} C_2H_5 \\ C_2H_5 \end{matrix}\!\!>\!C\!<\!\!\begin{matrix} CO.\,NH \\ NH.\,CO \end{matrix}\!\!>\!CH_2$$

Diethylmalonylcarbonyldiurea

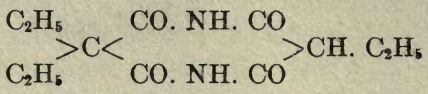
$$\begin{matrix} NH.\,CO.\,NH.\,CO \\ | \qquad\qquad\quad | \\ CO \qquad\qquad\; C \\ | \qquad\qquad\quad | \\ NH.\,CO.\,NH.\,CO \end{matrix}\!\!<\!\!\begin{matrix} C_2H_5 \\ C_2H_5 \end{matrix}$$

Dipropylmalonylmalonamide[3]

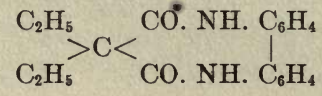
$$\begin{matrix} C_3H_7 \\ C_3H_7 \end{matrix}\!\!>\!C\!<\!\!\begin{matrix} CO.\,NH.\,CP \\ CO.\,NH.\,CO \end{matrix}\!\!>\!CH_2$$

[1] *Annalen*, 1908, **359**, 145.
[2] Rosenmund (*Ber.*, 1909, **42**, 4470).
[3] Remfry (*Trans.*, 1911, **99**, 618).

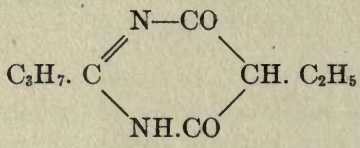
Diethylmalonylethylmalonamide[1]



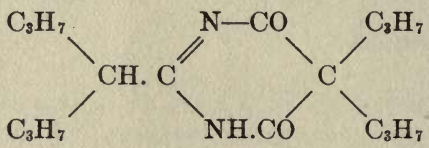
Diethylmalonylbenzidine[1]



4:6-*diketo*-2-*propyl*-5-*ethyltetrahydropyrimidine*[1]



4:6-*diketo*-5:5-*dipropyl*-2-∝-*propylbutyltetrahydropyrimidine*[1]



This last substance is a good example to show that multiplicity of alkyl-groups is of no avail when the nucleus of the compound is incorrect. In all these cases however, inactivity may be due to a possible insolubility in lipoid substance in the light of the Overton-Meyer hypothesis.

Many compounds described in recent literature have been prepared evidently (from their formulae) on the chance of their possessing hypnotic properties, but no reference can be found to the results of physiological tests. In all such cases we are forced to conclude that the substances were either inactive or had such small activity that it was of no use pursuing that course further. Belonging to this class are a large number of compounds pre-

[1]Remfrey (*Trans.*, 1911, **99**, 618)

pared by Clemmensen and Heitman,[1] all containing two ethyl radicles attached to one carbon atom and of the general formulae

$R_2 : C(OH)\ CO.\ NH$ $\quad$ $R_2 : C(OH)\ CO.\ NH.\ CO.\ NH_2$

$>CO$ and

$R_2 : C(OH)\ CO.\ NH$ $\quad$ $(R = C_2H_5)$

They also prepared diethylthiohydrantoin, which, however, would only be expected to have the same slight hypnotic power as diethylhydrantoin itself, and at the same time to be more toxic.

An example of the small structural change which is sufficient to deprive a substance of all hypnotic properties is afforded in the urethane as well as in the barbituric acid series.

Acetyl urethane (VII) which is as active as urethane itself, only differs materially from tertiary amyl allophante (VIII) in having a CH_3 in place of an NH_2 group, yet, whilst the former is possessed of moderate hypnotic powers, the latter is a perfectly inert substance.

Et. O. CO. NH. CO. CH_3 (VII) $\quad$ $(Me)_2$. Et C. O. CO. NH. CO. NH_2 (VIII)

It is curious that ethyl cinnamoylcarbamate $C_6H_5.CH{:}CH.\ CO.\ NH.CO_2C_2H_5$, where the NH_2 group of urethane is substituted by an aromatic in place of a fatty acid radicle, should prove quite inactive. Urethanes have not received much attention of late years; a great number of derivatives have already been prepared and no very satisfactory results having been obtained, has made it apparent that this substance is not a favourable groundwork to build on and hence it has been shelved for more promising subjects. A single exception to this is found in amylenehydrate-carbamate

$$(Me)_2\ Et\ C.\ O.\ CO.\ NH_2$$

which has been found[2] to have about twice the hypnotic power of amylenehydrate itself and to give good results where no very drastic treatment is necessary. This substance affords another example of the fact that alkyl-groups alone do not necessarily

[1] *Am. chem. journal*, 1908, **40**, 280.

[2] Huber (*Med. Klinik.*, 1911, 1234).

endow a compound with hypnotic properties as can be seen by comparing its formula with that of tertiary amyl allophanate.

Besides urethanes other acid amides are deprived of their hypnotic properties when substituted in the amido-group.

Ethyl cinnamoylcarbamate can be regarded as a substituted cinnamamide and in this case we have another active acid amide neutralised by the entrance of a carbonic ester into the amido radicle. It was shown many years ago[1] that most aromatic acid amides possess hypnotic properties in varying degree, but if one or both H atoms of the amido-group are substituted by an alkyl radicle then the substance becomes more like ammonia and strychnine in its action. Now, however, it has been demonstrated that other substituents as well as alkyl-groups are capable of depriving aromatic acid amides of their hypnotic properties, and it seems as if almost any substituent in the NH_2 group were sufficient for that purpose.

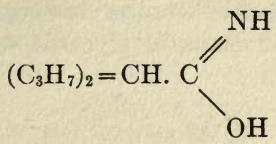
Carbonyl dicinnamamide (Dicannamoyl carbamide) $(C_6H_5.CH : CH.CONH)_2 = CO$ is quite inactive.[2]

A like result was found when the two active hypnotics cinnamamide and acetophenone are combined in cinnamoyl-p-aminoacetophenone $C_6H_5.CH : CH.CO.NH.C_6H_4.CO.CH_3$; also in the combination of bromisovaleramide, which has narcotic properties,[3] and acetophenone.[4] These compounds also afford further proof of the fact observed by Hildebrandt[5] that when p-aminoacetophenone was combined through the NH_2 group with an aldehyde, the hypnotic power was decreased unless the aldehyde possessed a free hydroxyl group, when an increase was observed.

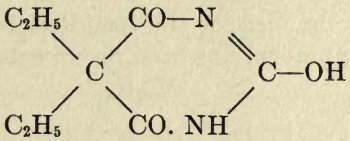
Fuchs[6] has advanced the theory that the presence of an OH group, as well as alkyl radicles, is necessary in an hypnotic substance in order to act as an anchor. This conclusion is arrived at in consideration of the fact that whilst diethyl-, ethylpropyl-

[1]Nebelthau (*Arch. expt. Path. Pharm.* 1895, **36**, 451).
[2]Remfry (*loc. cit.*).
[3]Eckhart (*Arch. expt. Path. Pharm.* 1907, **57**, 339).
[4]Remfry (*loc. cit.*).
[5]*Arch. expt. Path. Pharm.* 1905, **53**, 87.
[6]*Zeit. f. angew Chem.* 1904, **17**, 1505.

and dipropyl- etc., ketones are inactive the corresponding ketoximes are occasionally strong hypnotics. Also dipropylacetamide, which on being dissolved in alkali goes into its tautomeric iminoether form



is found to be more powerful in its action than such substances as chloral hydrate, sulphonal, amylenehydrate, etc., and is only surpassed by diethylbarbituric acid. This latter is also considered to act in the form



If this theory of the anchoring hydroxyl-group is correct it accounts satisfactorily for the loss of hypnotic properties by aromatic acid amides when both H atoms of the amido-group are replaced by alkyl radicles, it being impossible for the tautomeric iminoether form to be assumed. The acceptance of this theory moreover forces one to the conclusion that only in the case where no substitution at all occurs in the NH_2 group can a change to the tautomeric form take place, otherwise it is probable that some at least of the mono-substituted acid amides would have exhibited hypnotic properties.

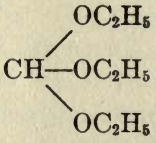
Dipropylacetbromamide $(C_3H_7)_2$ = CH. CO. NH. Br[1] is a further case in point. This compound is quite inactive whilst bromdipropylacetamide $(C_3H_7)_2$ = CBr. CO. NH_2 possesses considerable activity, only slightly less than that of the diethyl-compound which has found practical application.

Although having no close connection with acid amides it is of interest to note several cases which have appeared lately to show

[1]Fuchs (*loc. cit.*).

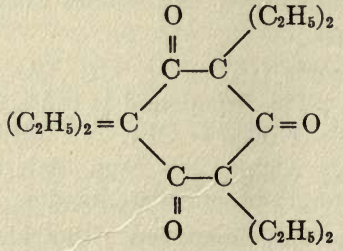
the varying results obtained by the introduction of alkyl-groups into a compound. Glycerol is quite inactive, as is also the trialkyl ether where the three alkyl groups are similar. When, however, one differs from the other two, or when all three are dissimilar, then it is claimed that substances having hypnotic properties are produced[1] as for example glycerin-α-ethyl-α-propyl-β-methylether.

Ortho ketone ethers of the general formula $\begin{matrix} R_1 & OC_2H_5 \\ >C< & \\ R_2 & OC_2H_5 \end{matrix}$ were made by Reitter and Hess,[2] which from the number of alkyl radicles and the general resemblance to ethyl ether might well be expected to have proved successful, yet turned out to be entirely without physiological action whatever. This appears somewhat strange in view of the fact that trioxyethylmethane (ortho formic ethyl ether) has been recommended



by Chevalier[3] as an antispasmodic where it evidently acts as a sedative or very mild hypnotic. The inactivity of the former compound may however, be due to insolubility in lipoid substance (Overton-Meyer theory).

A further example is given by Fränkel[4] who showed that the introduction of ethyl radicles into phloroglucin to the fullest extent, i.e., hexaethylphloroglucin, was without power to endow this substance



[1] D. R. P. 226, 454.
[2] *Ber.*, 1907, **40**, 3024.
[3] *Rep. de pharmacie*, 1907, **6**, 271.
[4] *Arch. expt. Path. Pharm.*, 1908, **58**, 181.

with narcotic properties, a compound being obtained having only a strychnine-like action.

In conclusion it may be mentioned that a close connection seems to exist between narcotics and local anaesthetics.

Gros[1] has compared the action of these two classes of substances and concludes that, to all intents and purposes, it is the same in both cases. He therefore considers that local anaesthetics are nothing more than strong hypnotics. For the purposes of comparison the narcotics employed were chloroform, paraldehyde, chloral, amylene hydrate, ethyl propionate, amylacetate, acetophenone, phenyl and ethyl urethane, whilst the local anaesthetics comprised alypin, cocaine, eucaine, stovain, tropacocain, nirvanin, holocain and subcutin.

Chloral has also been used in medical practice as a general anaesthetic but was found to be dangerous. Lately, however, another substance which, like chloral, was first employed as a hypnotic has been very successfully used in producing general anaesthesia. This substance is methylpropylcarbinolurethane, which was first introduced as a hypnotic about the year 1900. In 1910 its use as a general anaesthetic by intravenous injection

$$Me\ Pr\ CH.\ O.\ CO.\ NH_2$$

was first described,[2] since when several reports have appeared, the latest being by Page.[3]

It is therefore possible to attack the problem of the true manner in which hypnotics act from two points, and the fact that hypnotics also act as local and general anaesthetics irrespective of their volatility may help to elucidate the matter.

Finally, we should like to express our best thanks to Drs. H. H. Dale and P. P. Laidlaw of the Wellcome Physiological Research Laboratories, who have co-operated with us throughout the work and conducted most of the physiological experiments herein recorded.

[1] *Arch. expt. Path. Pharm.*, 1910, **62**, 380; 1910, **63**, 80; 1911, **64**, 67.
[2] Sichkovski (*Russki Vrach St. Petersbury* 1910, **9**, 1447).
[3] *Lancet*, 1012, **182**, 1258.

SUR LES ELEMENTS MINERAUX CONTENUS DANS LA CASEINE DU LAIT

Par M. L. Lindet

Paris

Nous connaissons bien mal l'état dans le quel se présentent certains éléments minéraux, quand nous les rencontrons associés à des matières protéiques. On dit communément par exemple que la caséine du lait renferme du phosphate de chaux, parceque l'analyse permet d'y déceler du phosphore et du calcium; dans la caséine précipitée par la présure, le phosphore, exprimé en $P_2 O_5$, représente de 3,50 à 3,55 % de la caséine sèche, alors que le calcium, exprimé en CaO, représente de 3,10 à 3,80%; si ces éléments formaient, à l'intérieur de la molécule protéique, du phosphate de chaux, celui-ci aurait une formule intermédiaire entre le phosphate bicalcique et le phosphate tricalcique.[1]

Je voudrais démontrer, dans ce mémoire, qu'une partie seulement du phosphore, environ la moitié, est à l'état de phosphate, probablement tricalcique, et que l'autre est engagée, à l'état d'acide phosphorique encore, dans une combinaison, hydrolisable par les alcalis. Quant à la chaux en excès par rapport à celle qui forme le phosphate de calcium, elle sature la fonction acide de la caséine; mais cette saturation n'est que partielle; car, comme je l'indiquerai plus loin, on peut faire absorber à la caséine, plus de 7% de chaux, comme on peut lui faire absorber de l'alumine, du zinc, etc. Il est probable que le phosphate de calcium est lui-même dissous par cette fonction acide; nous avons, M. L. Ammann et moi, (Ann. de l'Institut national agronomique, 1906, p. 283) montré que l'on peut saturer du

[1]Les dosages d'acide phosphorique et de chaux ont toujours été obtenus en attaquant la caséine par l'acide nitrique fumant, puis par l'acide sulfurique jusqu'à décoloration; on reprenait ensuite par l'eau et par l'ammoniaque; on acidulait par l'acide acétique, pour éliminer cusuite la chaux au moyen de l'oxalate; puis on dosait l'acide phosphorique à l'état de phosphate ammoniaco-magnésien.

caséinate de chaux par l'acide phosphorique sans que le liquide se trouble, c'est à dire sans que le phosphate formé se dépose. Il est également possible que le phosphate de chaux soit soluble dans le caséinate de chaux, bien que je n'aie pu jusqu'ici réaliser cette solubilisation, en partant du phosphate précipité; car il convient de remarquer que la caséine, précipitée, par la présure, est entièrement soluble, sans dépot de phosphate de chaux, dans l'ammoniaque et même dans la résorcine concentrée.

I. Je traite la caséine précipitée par la présure au moyen d'une solution acétique faible, et j'enlève de cette façon la chaux combinée à la fonction acide, et le phosphate de chaux, et j'obtiens un résidu décalcifié, qui renferme encore à peu près la moitié du phosphore que la caséine contenait primitivement.[1]

Les résultats de l'épuisement acétique de la caséine, provenant de l'emprésurage, sont consignés dans le tableau suivant:

POUR CENT DE CASÉINE SUPPOSÉE SÈCHE:

			ce qui représenterait:	
	P_2O_5	CaO	Phosphate de ℘ chaux	Chaux en excès enlevée par l'acide acétique
1er Épuisement	1,01	2,47	2,20	1,28
2e Épuisement	0,40	0,79	0,85	0,34
3e Épuisement	0,14	0,33	0,30	0,17
4e Épuisement	0,05	0,10	0,10	0,05
Résidu	1,88	0,00	0,10	0,05
	3,48	3,69	3,45	1,84
au lieu de	3,55	3,80		

[1]Les liquides acétiques dissolvent malheureusement de la caséine; on les en débarassait au moyen de sulfate de bioxide de mercure, et dans les liquides, additionnés de citrate d'ammoniaque et d'ammoniaque, on ajoutait le chlorure de magnésium; on s'assurait que le précipité mercurique ne renfermait pas d'acide phosphorique, en le reprenant par l'acide nitrique fumant.—J'ai obtenu également l'élimination de la caséine dissoute en chauffant les liqueurs en autoclave, en présence du formol.

Si tout le phosphore de la caséine s'y trouvait à l'état de phosphate de chaux, il n'y aurait aucune raison pour que l'acide étendu ne l'enlève pas en même temps que toute la chaux; quand on attaque en effet du phosphate tricalcique par de l'acide acétique éten du, l'acide phosphorique et la chaux se dissolvent, à tout moment, en quantités équivalentes. Nous dirons donc que l'acide acétique a fait disparaitre le phosphate de chaux (3,45% de la caséine) et la chaux combinée à la fonction acide de la caséine (1,84%).

La substitution de l'acide acétique à la présure dans la coagulation de la caséine détermine la précipitation d'une caséine pauvre en chaux, que l'on peut appauvrir davantage par un lavage à l'acide étendu; mais comme dans le cas ci-dessus, il reste du phosphore insoluble dans l'acide acétique étendu; celui-ci, compté en $P_2 O_5$, a représenté, dans mes expériences, sensiblement le même chiffre que précédemment (de 1,80 à 2,00%). Le fait est d'ailleurs connu des fabricants de caséine, qui, suivant l'usage au quel est destiné le produit, caillent le lait écrèmé, soit par la présure, soit par l'addition d'un acide minéral, soit par l'action biologique du ferment lactique; j'ai trouvé dans le commerce une caséine, provenent de l'acidification lactique, qui conservait encore une quantité de phosphore, représentant 1,80% de $P_2 O_5$.

J'ai été d'ailleurs à même de vérifier ce fait, en recherchant l'action de l'acide phénique sur le lait; je pensais que cet acide phénique, dont j'ai montré les propriétés, dissolvantes vis à vis de la chaux (Bin Soc. chimique. 1910, P. 435) serait capable de déplacer la chaux combinée à la fonction acide de la caséine; l'expérience a été négative. Mais elle n'a pas été inutile; car elle confirme ce qui vient d'etre dit: Deux portions d'un même lait, dont l'une avait été additionnée d'acide phénique, ont été caillées par la présure, et l'on a récolté les sérums; le lendemain, on a coagulé par la chaleur chacun d'eux, et on a dosé l'acide phosphorique et la chaux dans les coagulums et dans les liquides. Dans le coagulum du sérum phéniqué, il y a eu plus d'acide phosphorique et plus de chaux que dans le coagulum du sérum témoin, parceque ce sérum s'était acidifié du jour au lendemain, et que l'acide lactique produit avait enlevé du phosphate de

chaux et de la chaux; le complément de ces deux éléments se retrouve dans les liquides séparés du coagulum ainsi que le montre le tableau suivant:

		Rapporté au litre de lait : en grammes : P_2O_5	CaO
Coagulum	du sérum témoin	0,263	0,304
	du sérum phéniqué	0,279	0,349
Liquide séparé du coagulum	du sérum témoin	0,713	0,284
	du sérum phéniqué	0,700	0,245
Total dans le sérum primitif	témoin	0,976	0,588
	phéniqué	0,979	0,594

II. Pour rechercher l'état chimique que le phosphore affecte dans le résidu insoluble, j'ai eu recours, comme je l'ai dit plus haut, à une hydrolise ménagée en présence des alcalis ou des alcalino-terreux, et j'ai été frappé tout d'abord de la facilité avec la quelle ceux-ci dissocient, même à froid, la molécule de caséine. Mais ce qui nous intéresse en l'espèce, c'est que le phosphore de la caséine, qui restait insoluble dans l'acide acétique étendu, est dès lors facilement décelé à l'état d'acide phosphorique.

Si, par exemple, on traite par un lait de chaux de la caséine décalcifiée, et si on filtre, on obtient une solution qui renferme de la caséine, du phosphate de chaux et de la chaux en excès, et qui représente, comme nous l'avons appelé, M. L. Ammann et moi (loc. cit.) une solution de phosphocaséinate de chaux. Cette chaux en excès, abstraction faite de la chaux que l'eau dissoudrait naturellement, a représenté, dans mes expériences, de 7,30 à 7,75% de la caséine; elle est fixée par la fonction acide de la caséine. En outre, cette solution qui se décompose, qui se dégrade, en fonction du temps et de la température, donne naissance à de l'ammoniaque et aux produits que Schutzemberger a isolées, en chauffant des matières albuminoides à 180° en présence de la Baryte. L'addition d'acide acétique en excès dans une semblable solution précipite de la caséine non décomposée, en quantité d'autant plus grande que la dégrada-

tion a été moins accentuée. Mais ce qui frappe surtout, c'est que cette caséine ne renferme plus de phosphore, et que le phosphate de chaux, dissous dans l'acide acétique étendu, est passé dans les liqueurs. Les chiffres du tableau suivant indiquent la marche du phénomène:

	Durée du contact de la chaux avec la caséine	Caséine non dégradée % (1)	Caséine dégradée %	Azote de l'ammoniaque dégagée % de l'azote total	Acide phosphorique contenu dans la caséine précipitée
A 20-25°	24 heures	79,2	20,8	"	0
	48 heures	75,9	24,1	2,03	0
	96 heures	69,2	30,8	3,06	0
	10 jours	60,4	39,6		0
A 35°	48 heures	63,2	36,8	"	0

La soude ne dégrade pas et n'hydrolise pas l'acide phosphorique aussi vite que la chaux; quand on a soin de ne mettre que la quantité nécessaire de soude pour dissoudre une caséine à 1,80% d'acide phosphorique, on obtient, dans trois précipitations successives à l'acide acétique, des caséines qui renferment encore 1,66, 1,06, 0,78% d'acide phosphorique. A chaud, la dégradation de la caséine est plus rapide, et après un chauffage d'une heure à 120°, on ne précipite plus de caséine par l'acide acétique.

L'ammoniaque est, vis à vis de la caséine, encore moins énergique que la soude, et j'ai pu, en dissolvant à froid de la caséine à 1,80% d'acide phosphorique, avec le minimum d'ammoniaque, et pendant le minimum de temps, et en précipitant trois fois par l'acide, obtenir le même taux d'acide phosphorique. Mais un chauffage de cinq heures au Bain-Marie a fourni une caséine précipitée, qui ne renfermait plus que 1,30 d'acide phosphorique.

Je reviens à l'action de la chaux: On peut mettre, en évidence d'une façon plus élégante, cette action dissolvante de la chaux vis à vis de l'acide phosphorique que la caséine retient. La solution de phosphocaséinate de chaux, telle qu'elle a été préparée plus haut, est chauffée en autoclave, à 120°, pendant une heure;

(1) J'ai compté comme caséine non dégradée celle qui était précipitée par l'acide en excès, et celle que l'acidité acétique dissolvait normalement dans le liquide.

la dégradation de la matière protéique se produit; mais la caséine non dégradée, qui représente, dans ce cas, de 25 à 38% de la caséine primitive, se coagule, emprisonnant tout le phosphate primitif (P_2O_5 = 3,66% du coagulum) et un excès de chaux (CaO = 11,70% du coagulum), tandis que les liqueurs, qui renferment les matières azotées dégradées, en même temps que la chaux en excès, sont exemptes de phosphore. J'ai mesuré la dégradation de la caséine, dans ce cas, en dosant l'ammoniaque dégagée;[1] celle-ci, comptée en azote, a représenté de 13,5 à 26,0% de l'azote total.

Ce coagulum a été alors épuisé par de l'acide acétique étendu qui a enlevé très facilement le phosphate de chaux formé et la chaux en excès, en sorte qu'il est resté, comme le montre le tableau ci-dessous, une caséine sans calcium ni phosphore; cette caséine comme la précédente, se dissout dans la chaux, renferme 15,55% d'azote, etc.

POUR CENT DE CASÉINE SUPPOSÉE SÈCHE:

				ce qui représenterait:	
		P_2O_5	CaO	Phosphate Chaux	Chaux en excès, enlevée par l'acide acétique
1.	Épuisement	3,16	10,80	6,90	7,36
2.	Épuisement	0,30	0,54	0,65	0,19
3.	Épuisement	0,04	0,11	0,10	0,05
	Résidu	0,00	0,01	"	"
		3,50	11,46	7,65	7,60
	au lieu de	3,66	11,70		

Le fait que nous ne pouvons isoler le phosphore à l'état d'acide phosphorique sans dégrader la caséine mise en oeuvre, constitue-t-il une objection sérieuse contre le préformation de cet acide phosphorique dans la molécule de caséine? Je ne le crois pas. La dislocation de la matière protéique, dans la réaction de Schutzemberger, se produit sans oxydation; nous avons eu re-

[1]Le ballon était muni d'un tube à boules, contenant de l'acide sulfurique titré; de plus les liquides du ballon étaient saturés par de l'acide acétique; puis l'ammoniaque en était chassée en présence de Magnésie.

cours à une réaction moins énergique encore, puisque nous l'avons produite à la température ordinaire; dire que les réactifs employés ont été de nature à oxyder le phosphore métalloïdique équivaudrait à conclure que dans la lécithine, dans la phytine, etc., le phosphore peut n'etre pas à l'état d'acide phosphorique, puisque c'est par une saponification que l'on en sépare celui-ci. J'admets donc que, dans ces expériences, le phosphore qui a été retiré par l'action des alcalis se trouvait, préalablement à tout traitement, sous forme d'acide phosphorique.

J'ai à plusieures reprises cherché à réaliser cette sorte de saponification sous l'influence des seuls éléments contenus dans la caséine. Puisqu'une partie de la chaux de la caséine est combinée à sa fonction acide, ne peut-on pas, en faisant bouillir du lait, détacher cette chaux de l'acide faible que représente lacaséine, et la porter sur la molécule phosphorique saponifiable? Pour celà, je traitais du lait cru et du lait bouilli, puis refroidi, par une même quantité d'acide acétique; celui-ci dans le premier cas, devait dissoudre les phosphates naturels du lait, ainsi que le phosphate de chaux de la caséine, et, dans le second cas, en outre de ces phosphates, le phosphate de chaux formé par saponification. Je n'ai réussi qu'incomplètement, à cause de la faible alcalinité du lait; mais j'ai toujours eu, avec le sérum du lait cuit, plus d'acide phosphorique qu'avec le sérum du lait cru, ainsi que le montre le tableau suivant:

	Acide phosphorique dosé dans le sérum (en grammes)		Acide phosphorique du lait cuit pour un d'acide phosphorique du lait cru
	du lait cru	du lait cuit	
I	0,870	0,930	1,07
II	1,240	1,436	1,16
III	1,051	1,111	1,06
IV	1,106	1,260	1,14

III. La caséine que l'on précipite par la présure n'est pas la seule matière albuminoïde que l'on puisse extraire du lait; quand on chauffe le sérum qui s'égoutte de l'emprésurage, on obtient une matière albuminoïde qui semble, d'aprés les résultats que M. L. Ammann et moi avons fait connaitre (loc. cit.) un mélange de caséine et d'albumine. Le coagulum renferme du

phosphore, qui, compté em P_2O_5, représente de 4,86 à 6,17%, et du calcium, qui, compté en CaO, représente de 5,71 à 7,52%. Il est donc plus riche en éléments minéraux que la caséine provenant directement de l'emprésurage. J'ai épuisé également ce coagulum par l'acide acétique étendu; mais il est resté, comme dans le cas précédent, du phosphore non dissous:

POUR CENT DE CASÉINE SUPPOSÉE SÈCHE:

	P_2O_5	Cao	ce qui représenterait: Phosphate de Chaux	Chaux en excés, enlevée par l'acide acétique
1. Épuisement	3,92	5,43	8,55	0,80
2. Épuisement	1,27	1,84	2,75	0,36
3. Épuisement	0,29	0,30	0,60	0,00
Résidu	0,73	0,00	"	"
	6,21	7,57	11,90	1,16
au lieu de	6,17	7,52		

Je n'ai pu appliquer à ce coagulum épuisé par l'acide acétique étendu la méthode que j'ai décrite plus haut pour en extraire le phosphore résiduaire à l'état d'acide phosphorique, parceque la matière, qui avait été coagulée par la chaleur ne se redissolvait qu'incomplètement dans un lait de chaux.

IV. J'ai voulu substituer à l'acide acétique étendu pour la dissolution du phosphate de chaux et de la chaux en excès dans la caséine d'emprésurage, le citrate d'ammoniaque ammoniacal. Ce réactif a laissé dans le résidu insoluble, une quantité de phosphore inférieure à celle que l'acide acétique a laissée; mais il convient de remarquer que l'on agit en milieu alcalin, et que l'alcali est capable de saponifier une partie de l'acide phosphorique, comme le fait la chaux:

POUR 100 DE CASÉINE SUPPOSÉE SÈCHE:

	P_2O_5	CaO	ce qui représente: Phosphate de Chaux	Chaux en excés, enlevée par l'acide acétique
1er Épuisement	1,26	2,40	2,75	0,91
2e Épuisement	0,36	0,72	0,80	0,28
3e Épuisement	0,33	0,23	0,70	0,00
4e Épuisement	0,21	0,10	0,45	"
5e Épuisement	0,13	0,03	0,15	"
Résidu	0,85	0,00	"	"
	3,14	3,48	4,85	1,19
au lieu de	3,85	3,80		

C'est encore cette action saponifiante de l'ammoniaque qui permet d'expliquer le fait suivant: Quand on cherche à précipiter, en présence de caséine, par exemple dans du lait écrémé, l'acide phosphorique à l'état de phosphate ammoniaco-magnésien, on n'obtient, au bout de 24 heures, que 30% environ du phosphore contenu dans la caséine ou dans le lait; la caséine gène la précipitation; mais celle-ci se continue lentement, au fur et à mesure que la caséine se dégrade en produits moins visqueux et que la combinaison phosphorique se saponifie, et au bout de six mois on peut receuillir jusqu'à 81,9% du phosphore total, alors que 50% environ était, dans la caséine primitive, à l'état de phosphate de chaux.

Ce phénomène semble dépendre, non de la quantité de caséine dissoute dans la liqueur, mais du rapport de l'acide phosphorique dissous à la caséine dissoute; car, en précipitant une même liqueur, concentrée ou étendus d'eau et d'ammoniaque, de façon à avoir la même quantité d'alcali, j'ai obtenu, après le même temps, la même quantité de phosphate ammoniaco-magnésien.

Nous conclurons donc de cette étude que l'acide phosphorique et la chaux forment trois groupes d'éléments minéraux: de la chaux combinée à la fonction acide, du phosphate de chaux, probablement tricalcique, et de l'acide phosphorique, retenu par la molécule protéique, et susceptible d'en etre détachée par hydrolise ou saponification.

L'étude du soufre contenu dans la molécule de caséine fera l'objet d'une étude ultérieure.

(*Extrait*)

LA QUESTION DE L'ACIDE SULFUREUX DANS LES VINS BLANCS

Par Philippe Malvezin

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L'auteur frappé par les contradictions qui existent entre les résultats des recherches sur l'action physiologique de l'acide sulfureux contenus dans les vins blancs, entreprises à Bordeaux par une commission d'étude nommée à cet effet, et les conclusions du rapport présenté par M. le Prof. Gautrelet, rapporteur, a repris l'examen détaillé des tableaux d'expériences et a pu établir ainsi, dans sa communication en reproduisant des tableaux comparatifs formés des chiffres pris à même le rapport de M. Gautrelet; en soulignant, d'autre part, certains passage des commentaires d'expériences, que les conclusions du rapport de Bordeaux ne sont *nullement celles qui découlent naturellement des expériences* dont l'auteur analyse l'essence au cours de sa communication.

En se basant sur les résultats purement expérimentaux de la commission bordelaise, l'auteur en arrive à conclure que les expériences de Wiley semblent bien plutôt *confirmées* qu'infirmées par le rapport, et il émet le voeu que *l'usage de l'acide sulfureux dans les vins soit étroitement réglementé en attendant qu'il ait été établi par une commission internationale de chimistes et de médecins, quelles sont les doses de cet antiseptique qui peuvent être tolérées par tous les organismes humains et pendant une longue durée correspondant à une absorbtion habituelle de vin sulfité.* (Les expériences de Bordeaux sont une heureuse initiative, sans doute, mais incomplètes, écourtées et où l'auto-sugestion semble avoir joué un trop grand rôle.)

(La santé publique et le commerce français trouveront l'un et l'autre leur compte à ce qu'il ne puisse plus être émis de doute à l'égard de nos produits nationaux.)

THE INFLUENCE OF HYDROXYL AND CARBOXYL GROUPS ON THE PHARMACOLOGICAL ACTION OF NITRIC ESTERS

By C. R. Marshall, M. D.
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As a pharmacological group the nitric esters belong to the class of vaso-dilators. Their chief action is exerted on unstriped muscle fibre, and especially on that of the blood vessels. Nearly all the nitric esters so far investigated cause a fall of blood-pressure owing to dilatation of the arterioles, and when administered in small doses this is almost their sole effect. This action, consequently, forms a convenient test for determining the pharmacological activity of any member of the group, and it has been the one employed in this investigation. The experiments were made on anæsthetised rabbits and cats. The blood-pressure was taken from the common carotid artery. The injections were made into one of the facial veins (rabbits) or into the external jugular vein (cats).

The substances employed in the investigation were: glycerol-dinitrate, methyl-glycerol-dinitrate, tetra-methyl-mannitol-dinitrate, di-methyl-mannitol-tetranitrate, mannitol-pentanitrate, ducitol-pentanitrate, and the nitric esters of tartaric and ehtyl-tartaric acids, of citric and ethyl-citric acids, and of lactic and ethyl-lactic acids. The glycerol-dinitrate was prepared according to the method of Will;[1] the mannitol-pentanitrate and dulcitol-pentanitrate were obtained by reducing the corresponding hexanitrates by means of pyridin;[2] the remainder were made by nitrating in the ordinary way the corresponding alcohols, acids or alkyl compounds by means of a mixture of nitric and sulphuric acids kept cool by a freezing mixture.[3] As

[1]Ber. XXXXI p. 1107 (1908).

[2]Wigner, Ber. XXXVI p. 794 (1903).

[3]I am indebted to the kindness of my colleague Professor Irving for the methyl-glycerol, Di-methyl-mannitol, and Tetra-methyl-mannitol from which the nitric esters were made.

most of the nitric esters are but slightly soluble in water, diluted alcohol was frequently used to prepare the injections.

The presence of hydroxyl or methoxyl groups appears to diminish very considerably the vaso-dilating action of this group of substances. Glycerol dinitrate and methyl-glycerol dinitrate, for example, are much less powerful than nitroglycerine, and the loss of effect with increase of methoxyl groups is even more marked in the compounds of mannitol. Thus in one experiment (exp. I) in which 0.01 g. glycerol dinitrate reduced the blood-pressure from 81 Mm. Hg. to 52 Mm. Hg., one-twentieth this dose of nitro-glycerin caused a fall from 80 Mm. Hg. to 58 Mm. Hg.; and in another experiment (exp. II) the dose of tetramethyl-mannitol dinitrate causing a minimal effect—a fall of 2-3 Mm. Hg. was found to be 0.002g., whereas a similar effect was produced by 0.0003g. of dimethyl-mannitol tetranitrate. This dose of mannitol pentanitrate, although not given in this particular experiment, produces a decided fall of blood-pressure. Obviously the effect is not merely due to the smaller number of nitrate groups since the loss of activity is much greater than this will explain.

When compared with completely nitrated alcohols containing the same number of nitrate groups, most of the esters containing a hydroxyl or methoxyl group are less active. The exception occurs in the case of the glycerol dinitrates which seem to be at least equal in activity to the glycol dinitric esters (glycol dinitrate, propylene-glycol dinitrate, trimethylene-glycol dinitrate) I have tried (exp. III). Tetramethyl-mannitol dinitrate, however, is less active than these, and dimethyl-mannitol tetranitrate is much less active than erythritol tetranitrate. Mannitol pentanitrate and dulicitol pentanitrate are also less active than arabitol pentanitrate or erythritol tetranitrate or glycerol trinitrate (exp. IV-VI).

The following experiments will serve to illustrate these remarks. To economize space the blood-pressure before the injection and the lowest blood-pressure reached after the injection are alone given. And for the same reason in most cases only a portion of the experiment is described. The series of injections given, however, is consecutive, the injections left out

being for the most part repetitions of the substances mentioned in different series or in different doses.

Exp. I. Rabbit. Ether

	Fall of Blood Pressure
1.0Cc. 1 per cent. Glycerol Dinitrate	from 81 to 52Mm.Hg.
0.5Cc. 0.1 per cent. Glycerol Trinitrate	from 80 to 58Mm.Hg.

Exp. II. Cat. 2850g. Chloroform then ether

	Fall of Blood Pressure
1Cc. 1/500 Methyl-glycerol Dinitrate	from 158 to 116Mm.Hg.
1c. 1/500 Tetramethyl-mannitol Dinitrate (partly suspended)	from 151 to 149Mm.Hg.
1Cc. 1/100 Tetramethyl-mannitol Dinitrate (in 29% alcohol; partly suspended)	from 150 to 128Mm.Hg.
1Cc. 1/3000 Dimethyl-mannitol Tetranitrate (in 12.5% alcohol, partly suspended)	from 142 to 139Mm.Hg.
1Cc. 1/50 Methyl-glycerol Dinitrate (in 14% alcohol, partly suspended)	from 137 to 72Mm.Hg.
1Cc. 1/600 Dimethyl-mannitol Tetranitrate (in 75% alcohol)	from 126 to 67Mm.Hg.
1Cc. 1/500 Glycol Dinitrate	from 130 to 101Mm.Hg.

Exp. III. Rabbit. 2000g. Ether

	Fall of Blood Pressure
1Cc. 1/500 Propylene-glycol Dinitrate	from 66 to 50Mm.Hg.
1Cc. 1/500 Glycol Dinitrate	from 62 to 50Mm.Hg.
1Cc. 1/500 Trimethylene-glycol Dinitrate (partly suspended)	from 62 to 51Mm.Hg.
1Cc. 1/500 Glycerol Dinitrate	from 65 to 50Mm.Hg.
1Cc. 1/500 Propylene-glycol Dinitrate	from 64 to 54Mm.Hg.

Exp. IV. Cat. 2950g. Ether

	Fall of Blood Pressure
1Cc. $\frac{1}{10000}$ Mannitol Pentanitrate (in 20% alcohol)	from 123 to 117Mm.Hg.
1Cc. $\frac{1}{10000}$ Glycerol Trinitrate (in 20% alcohol)	from 120 to 97Mm.Hg.
1Cc. $\frac{1}{10000}$ Erythritol Tetranitrate (in 20% alcohol)	from 120 to 105Mm.Hg.
1Cc. $\frac{1}{10000}$ Mannitol Pentanitrate (in 20% alcohol)	from 116 to 113Mm.Hg.
1Cc. $\frac{1}{10000}$ Arabitol Pentanitrate (in 20% alcohol)	from 112 to 101Mm.Hg.

Exp. V. Rabbit. 1450g. Ether

	Fall of Blood Pressure
1Cc. $\frac{1}{5000}$ Dulcitol Pentanitrate (in 20% alcohol)	from 54 to 49Mm.Hg.
1Cc. $\frac{1}{5000}$ Dulcitol Hexanitrate (in 30% alcohol)	from 49 to 35Mm.Hg.
1Cc. $\frac{1}{10000}$ Arabitol Pentanitrate (in 20% alcohol)	from 43 to 32Mm.Hg.
1Cc. $\frac{1}{5000}$ Dulcitol Pentanitrate (in 20% alcohol)	from 56 to 50Mm.Hg.
1Cc. $\frac{1}{5000}$ Mannitol Pentanitrate (in 20% alcohol)	from 52 to 44Mm.Hg.
1Cc. $\frac{1}{10000}$ Glycerol Trinitrate (in 20% alcohol)	from 48 to 34Mm.Hg.

Exp. VI. Rabbit. 2250g. Chloroform

	Fall of Blood Pressure
1Cc. $\frac{1}{3000}$ Mannitol Pentanitrate	from 76 to 54Mm.Hg.
1Cc. $\frac{1}{20000}$ Erythritol Tetranitrate	from 76 to 56Mm.Hg.
1Cc. $\frac{1}{3000}$ Glycerol Trinitrate	from 77 to 49Mm.Hg.
1Cc. $\frac{1}{3000}$ Mannitol Pentanitrate	from 78 to 58Mm.Hg.

The influence of the carboxyl group on the vaso-dilating action of nitric esters is still more marked than that of the hydroxyl group. The nitric esters of tartaric, citric, and lactic acids, neutralised with sodium bicarbonate, produced, when injected intravenously, no fall of blood-pressure whatever, and the nitric esters of methyl-citric and methyl-lactic acids caused a fall only after the lapse of several minutes.

Exp. VII. Rabbit. 1850g. Ether

	Fall of Blood Pressure
2Cc. $\frac{1}{10}$ Tartaric Acid Dinitrate (neutralised)	no effect
0.8Cc. $\frac{1}{20000}$ Erythritol Tetranitrate	from 80 to 47Mm.Hg.

Exp. VIII. Rabbit. 1850g. Chloroform

	Fall of Blood Pressure
1Cc. $\frac{1}{200}$ Ethyl-tartaric Acid Dinitrate (25% alcohol)	no effect.
1Cc. $\frac{1}{200}$ Ethyl-citric Acid Nitrate (10% alcohol)	no fall for 3 mins. then gradual fall from 98 to 72Mm.Hg. at 8 mins.
1Cc. $\frac{1}{200}$ Ethyl-lactic Acid Nitrate (25% alcohol)	no fall for 3 mins. then gradual fall from 87 to 68Mm.Hg. at 11 mins.
1Cc. $\frac{1}{3000}$ Mannito Pentanitrate	from 76 to 53 Mm.Hg.

THE PHARMACOLOGICAL ACTION OF BROM-STRYCHNINES

By C. R. Marshall, M. D.

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Three brom-strychnines have been described—two mono-derivatives and one di-derivative. The first monobromstrychnine, $C_{21}H_{21}O_2N_2Br$, was prepared simultaneously by Shenstone[1] and Bechurts[2] and was obtained by the action of equi-molecular proportions of bromine and strychnine hydrochloride (Shenstone) or hydrobromide (Bechurts) in aqueous solution. It forms rhombic crystals melting at 222° C. (Bechurts). Later Loebisch and Schoop[3] by the action of bromine on strychnine in strong sulphuric acid, obtained a product crystallising in needles arranged in rosettes and giving different colour reactions from those given by the monobromstrychnine of Shenstone and Bechurts. This substance they regarded as a new monobromstrychnine and termed it β monobromstrychnine to distinguish it from the monobromstrychnine previously obtained. Still later a monobromstrychnine was obtained by Martin[4] in colourless needles melting at 199° C. but no colour reactions of this substance are given.

Dibromstrychnine, $C_{21}H_{20}O_2N_2Br_2$, was first described by Bechurts[5] who obtained it, along with monobromstrychnine and apparently some perbromide, by the action of four atoms of bromine (as bromine water) on one molecule of strychnine hydrobromide in aqueous solution. It formed rhombic crystals, which when heated to 230° C. decomposed and gave off red brown fumes. Its solution in dilute alcohol when warmed on the water-bath quickly became acid; aldehyde and hydrobromic acid were

[1]Journ. Chem. Soc. XLVII p. 139 (1885).
[2]Ber. XVIII p. 1236 (1885).
[3]Monatsh. f. Chem. VI p. 855 (1885).
[4]Bull. Soc. Chim. de Paris (3) XXXI p. 386 (1904).
[5]Ber. XVIII p. 1237 (1885).

given off and monobromstrychnine formed. More recently Bechurts[1] has described this reaction of two equivalents of bromine on strychnine as resulting in the formation of monobromstrychnine hydrobromide and bromstrychnine dibromide. An excess of bromine produced monobromstrychnine tribromide. A year previous to this Martin[2] by a method similar to that used to prepare his monobromstrychnine obtained what he describes as a dibromstrychnine as small colourless crystals melting at 130-131° C. which became coloured on exposure to light. More recently Ciusa and Scagliarini,[3] by the action of bromine on strychnine in glacial acetic acid have obtained what they regard as strychnine dibromide which is said to exist in two modifications, an unstable form crystallising from alcohol in colourless needles united into rosettes, and a stable form obtained by repeated recrystallisations or better by fusion of the first form and differing from it in melting at 260° C. and crystallising in large monoclinic crystals. Like the dibromstrychnine of Bechurts it proved to be easily converted into monobromstrychnine.

In view of the unsatisfactory state of this subject it may be of interest to publish some pharmacological experiments on brominated products of strychnine made ten years ago.

Mono-Brom-Strychnines. The two monobromstrychnines were prepared according to the methods of Shenstone and of Loebisch and Schoop respectively, and, for purposes of pharmacological investigation, were converted into the hydrobromides. The bases crystallised in different forms and gave somewhat different colour reactions. Crystallised from hot absolute alcohol the α monobromstrychnine separate as large crystals, the β monobromstrychnine as amorphous globules. The latter, however, readily crystallised from hot water forming long prisms for the most part united into rosettes. When dissolved in concentrated sulphuric acid and a crystal of potassium bichromate was added the α variety showed a very transient blue colour passing quickly through green to a light brownish-yellow; the β variety gave a

[1]Arch. d. Pharmaz. CCXLIII p. 493 (1905).
[2]Bull. Soc. Chim. de Paris (3) XXXI p. 388 (1904).
[3]Atti del. Accad. dei Lincei (5) XIX p. 555 (1910).

more permanent and deeper blue which passed through purple to a light red.

Pharmacological. The bromstrychnine isolated by Shenstone was investigated pharmacologically by Lauder Brunton[1] who, however, merely states that " In the pithed frog it causes clonic convulsions, which, like those of strychnine, may be brought on by a slight touch, jar, or external irritation." Unfortunately the dose given is not mentioned. Loebisch and Schoop[2] after administering 0.0016g. ∝-monobromstrychnine (Bechurts) to a frog, observed increased sensitiveness in three minutes, tetanus in five minutes, and death in thirty minutes. After the same dose of β- monobromstrychnine they noticed at first diminution in reflex excitability, and after eight minutes fibrillary twitchings of the muscles. Tetanus occurred later and death followed thirty minutes after the administration.

My experiments show that both monobromstrychnines behave, pharmacologically, like a weak strychnine. Of the two the β compound seemed to be slightly the more powerful; it induced convulsions somewhat earlier than the ∝ compound, but in some cases these early convulsions were more transient than those obtained with the ∝ variety. I have not observed the preliminary diminution in reflex excitability described by Loebisch and Schoop with the β modification, nor do any of my experiments show, as their experiment does, the later appearance of convulsions in the β as compared with the ∝ compound.

The following table gives the time of onset, in minutes of the first convulsion with different doses (calculated to one gramme body-weight of frog) of the hydrobromide in Rana temporaria.

Dose	∝ -monobromstrychnine	*B* -monobromstrychnine
0.003Mg. p. g.	30′	15′
0.005Mg. p. g.		10′
0.006Mg. p. g.	13′	
0.05Mg. p. g.	5′	3′

Di-Brom-Strychnine(?) This substance was prepared for me by my friend H. A. D. Jowett, D.Sc. for the purposes of another re-

[1]Journ. Chem. Soc. XLVII p. 144 (1885).
[2]Monatsh. f. Chem. VI p. 861 (1885).

search, and was made by adding bromine to strychnine in glacial acetic acid, decomposing the perbromide with ammonia, and crystallising the precipitate formed from alcohol. The small almost colourless crystals have remained apparently unchanged for the last ten years. A Carius determination showed that it contained two bromine atoms, and one of these was found to be broken off by dissolving the substance in nitric acid and adding silver nitrate at ordinary temperatures so that the substance was probably monobromstrychnine bromide. Like the dibromstrychnine of Bechurts a solution in diluted alcohol heated on the water-bath acquired an acid reaction; but the change to monobromstrychnine must have been relatively slow since the product, after heating for half an hour, produced a pharmacological effect more closely resembling that of the parent substance than that of monobromstrychnine. The substance decomposed on heating and consequently had no definite melting point. When subjected to the ordinary strychnine reaction it gave a very transient purple passing into reddish-yellow.

Pharmacological. In the paper already cited, Ciusa and Scagliarini state that they have studied the physiological action of monobromstrychnine and the two strychnine dibromides described by them, but I have been unable to find any description of these experiments. And, as far as I am aware, the pharmacological action of a brominated strychnine containing more than one atom of bromine has not previously been described.

The substance I have investigated differs markedly from the monobromstrychnines in pharmacological action. It is not only much less toxic but it produces, in frogs at least, paralytic symptoms of peripheral origin. In rabbits no obvious paralysis was observed.

When 0.01Mg. per gramme body-weight was injected into the dorsal lymph sac of a grass frog there appeared in fifteen to twenty minutes, slight depression and the animal remained on its back for a short time when placed in that position. From this state of lethargy it gradually recovered without showing any other symptoms. After an injection of 0.025Mg. per gramme body-weight the animal commenced to sink on to the table in three minutes and when laid on the back was unable to turn over.

Gradual recovery from this state of paralysis occurred and then the animal developed a condition of increased excitability, a slight tetanic convulsion being produced by hitting the table. This condition was observed in one case on the following day. Still larger doses produce more obvious convulsive symptoms. After an injection of 0.05 Mg. per gramme body-weight into the dorsal lymph sac the animal manifested the same paralytic symptoms but six minutes after the injection slight twitches of the limbs were observed on hitting the table and a few apparently independent twitches of the toes occurred fifteen minutes after the administration. Otherwise the animal lay as if paralyzed. Four hours after the injection the frog had almost recovered, slight increased excitability being alone present.

To determine whether the paralytic symptoms were of central or peripheral origin the right thigh of a frog was ligatured and an injection of 0.3Mg. per gramme body-weight made into the dorsal lymph sac. Three minutes after the injection the animal commenced to sink on the table. Respiratory movements were still present but failed later. Six minutes after the injection the right limb become tetanic on touching any part of the body, the left limb merely gave a momentary twitch and then remained lax. This condition continued, except that on repeated stimulation the left limb often failed to twitch, until the frog was pithed fifteen minutes after the injection. The irritability of the cut sciatic nerves to electrical stimulation (one accumulator cell) was then determined, with the following result.

Left sciatic. Secondary coil 24—5Cm.
= slight contraction of toes.
Left sciatic. Secondary coil 0Gm.
= marked contraction of leg.
Right sciatic. Secondary coil 47Cm.
= decided contraction of leg.

Since the muscles reacted to weak stimulation it would seem from this experiment that the paralysis is due to depression of the nerve-endings.

As already stated no symptoms of paralysis were observed in rabbits. After injecting subcutaneously 5Mg. per kg. no unequivocal symptoms occurred. The animal became quieter after eight minutes and there was a slight fall in the frequency of the respirations and the heart beats. Forty minutes after the first injection a second injection of 15Mg. per kg. was given. This caused slightly increased reflex excitability which commenced eleven minutes after the injection and continued for about forty minutes. Twenty minutes after the administration a slight tetanic attack was produced by hitting the table. No other symptoms were noticed.

The pharmacological evidence would seem to show that, whatever the constitution of this supposed dibromstrychnine may be, both bromines form an integral part of the molecule. The difference in action between this substance and the monobromstrychnine hydrobromides can scarcely be explained on any other grounds. It is true that relatively slight modifications of strychnine, such as the formation of strychnine oxide or the conversion to isostrychnine, would produce similar effects, but such an assumption is unnecessary and it is improbable that such a change in the strychnine molecule would be brought about by the method used in preparing this substance. It is well known that various strychnine derivatives, e.g. methyl-strychnine, are predominently paralytic in action, and strychnine itself, in large doses, exerts a paralysing influence on the motor nerve-endings of frogs. To what portion of the strychnine molecule, if indeed any, this paralysing influence is due, has not been determined, but it is of interest to point out that in this so-called dibromstrychnine, it is mainly the convulsant action of strychnine which has been lost rather than a new action which has been acquired. In other words, strychnine, administered in the doses necessary in the case of the di-brominated compound, exerts a depressant action on motor nerve-endings.

In this connection it is also of interest to note that each bromine atom causes a uniform fall in the convulsant power of the substance. Thus the minimal dose per gramme body-weight of

frog, injected into the dorsal lymph sac, necessary to produce the slightest convulsive symptoms was found to be, for—

strychnine	0.00035Mg.
monobromstrychnine	0.003Mg.
dibromstrychnine(?)	0.025Mg.

Summary. The two monobromstrychnines act like strychnine but are 8–9 times weaker.

Dibromstrychnine(?), although it also possesses a convulsant action, produces in frogs mainly paralysis due to depression of the motor nerve-endings.

RELATIONS DE LA PLANTE AVEC LES ÉLÉMENTS FERTILISANTS DU SOL: LOI DU MINIMUM ET LOI DES RAPPORTS PHYSIOLOGIQUES

Par P. Mazé

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Le rendement d'une culture effectuée sur un milieu nutritif naturel ou artificiel se règle, dit-on, sur la substance alimentaire la moins abondante par rapport aux besoins de la plante.

Voilà l'énoncé d'une loi connue en agriculture sous le nom de *loi du minimum.*

Mes recherches sur le développements du maïs en milieu aseptique m'ont permis de constater que cette loi est une conception purement spéculative.

Les relations d'un végétal avec son substratum nutritif sont subordonnées, comme je vais le montrer, à des conditions multiples qui obéissent à une loi que j'appellerai la *loi des rapports physiologiques.*

Soit par exemple le milieu suivant, tableau I,

Phosphate de potassium neutre	1.	Chlorure de zinc	0.05
Sulfate de magnésium + 7 aq.	0.2	Silicate de potassium	0.05
Sulfate ferreux + 7 aq.	0.1	Carbonate de calcium	2.
Chlorure de manganèse + 4 aq.	0.05	Eau distillée	1000.

qui, additionné de 1 p. 1000 de nitrate de sodium ou d'un sel azoté de richesse équivalente en azote, constitue une solution nutritive dans laquelle le maïs accomplit son évolution complète jusqu'à la maturation des graines.

On y fait pousser des plantes jusqu'à ce qu'elles aient atteint un poids en moyen de 10 gr. A partir de ce moment on les place dans une solution incomplète pourvue d'un seul élément nutritif.

La plante vit alors sur les réserves de matières minérales qu'elle a empruntées à la solution mère. Dans ces réserves les divers éléments présentent entre eux les rapports les plus favorables au développement du végétal lui-même. L'élément en solution

vient troubler ces rapports; les chiffres du tableau II montrent jusqu'à quel point son influence s'exerce sur l'évolution de la plante, l'eau distillée étant prise comme terme de comparaison. Les poids de matières sèches sont déterminés au moment où la plante peut être considérée comme morte.

TABLEAU II

Nature de l'aliment azoté de la solution mère	Substance introduite dans la solution incomplète en gr. p. 1000.	Poids sec de la plante calculé au moment de l'introduction de la solution incomplète en gr.	Poids sec de la plante à la fin de l'expérience en gr.	Gain de matières sèches en gr.	Rapport du gain au poids sec initial	Durée de l'expérience en jours.
Nitrate d'ammonium	No_3NH_4-0,5	13,976	40,10	26,224	1,87	49
Nitrate de sodium	No_3Na-1	7,947	27,38	19,433	2,44	48
Sulfate d'ammonium	$So_4(NH_4)_2$-0,5	9,751	15,79	6,039	0,61	40
Chlorure d'ammonium	NH_4Cl-0,5	7,315	13,36	6,045	0,82	39
Nitrate de sodium	$Po_4HK_2$1	12,002	33,015	21,013	1,75	60
Nitrate d'ammonium	Eau distil.	10,084	39,65	29,566	2,94	47

Les substances minérales nutritives des solutions incomplètes, bien qu'offertes à des concentrations favorables au développement des plantes, c'est-à-dire à des concentrations physiologiques, arrêtent l'évolution du végétal parce que les rapports des divers éléments minéraux de la plante et de la solution ne répondent plus aux exigences de la nutrition de la cellule vivante. C'est l'eau distillée, où ces rapports ne sont pas altérés, qui donne les meilleurs résultats et de beaucoup.

. . . .

On peut procéder d'une autre manière pour mettre en évidence les influences des solutions incomplètes. Au lieu d'opérer avec des plantes déjà bien développées, on utilise des plantes qu'on a fait germer dans l'eau distillée.

On observe alors des résultats variés parmi lesquels les suivants seuls nous intéressent.

Toutes les solutions constituées par un seul élément nutritif qui ne renferme ni soufre ni fer provoquent une chlorose plus ou

moins intense du maïs. Les plantes placées dans l'eau distillée conservent leur couleur verte pendant toute la durée de l'expérience. Le nitrate de potassium à 0.5 p. 1000 décolore les feuilles de la plantule formées après l'immersion des racines dans la solution incomplète; on peut constater que l'élément soustrait à la plantule par voie d'exosmose est le fer; une solution de nitrate ferrique à 0 g. 2 p. 1000 fait apparaître la chlorophylle à l'endroit où des gouttelettes déposées sur le limbe des feuilles abandonnent aux cellules du parenchyme des traces de fer.

On voit ainsi que les solutions incomplètes agissent sur les plantes supérieures suivantes des procédés variés.

. . . .

Les végétaux inférieurs se prêtent très bien aussi à ces démonstrations.

Comme les divers éléments du liquide Raulin présentent entre eux des rapports physiologiques très favorables au développement de *l'aspergillus niger*, c'est ce dernier que j'ai utilisé.

J'ai déterminé d'abord les limites de concentration que l'aspergillus peut supporter, en le cultivant sur des solutions Raulin de concentration $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 4, 8, 16 dans lesquelles l'acidité et la teneur en sucres restent constantes et de même valeur que dans 1.

Le tableau III donne les résultats comparatifs de ces essais.

Durée des cultures à 30° 4 jours.

TABLEAU III

Concentration	$\frac{1}{4}$	$\frac{1}{2}$	1	2	4	8	16
Poids du mycélium en gr.	0 473	0.737	1.094	1.057	0.1960	0.842	0.872

Prenons maintenant les concentrations extrêmes $\frac{1}{2}$, 1 et 16, et portons dans $\frac{1}{2}$ ou 1, la concentration de l'élément Azote à 16, en multipliant le nitrate d'ammonium par le coefficient 16. Si les renseignements fournis par les végétaux supérieurs se confirment, les milieux $\frac{1}{2}$ + 16 Az. et 1 + 16 Az. donneront un poids de mycélium inférieur à celui qu'on récoltera sur les milieux $\frac{1}{2}$, 1 et 16, le sucre et l'acidité restant constants.

Les résultats sont consignés dans le tableau IV.

TABLEAU IV

	Durée des cultures en jours	Concentration	Poids du mycélium en grammes
	3	$\frac{1}{2}$	0.466
I.	3	$\frac{1}{2}$ + 16 Az.	0.217
	3	16	0.723
	4	$\frac{1}{2}$	0.646
II.	4	$\frac{1}{2}$ + 16 Az.	0.481
	4	16	0.830
	3	$\frac{1}{2}$	0.475
III.	3	$\frac{1}{2}$ + 16 Az	0.316
	3	16	0.991
IV.	3	1	0.758
	3	1 + 16 Az.	0.386
	3	16	0.972
	4	1	0.905
V.	4	1 + 16 Az.	0.495
	4	16	1.109

Ces résultats sont, comme on le voit, en contradiction avec *la loi du minimum* et obéissent à la *loi des rapports physiologiques.*

Dans la pratique agricole l'emploi irraisonné des engrais minéreaux solubles peut conduire à des abaissements de rendement, si l'on ne tient pas compte de ces données. Je préciserai d'ailleurs, bientôt, les règles qui doivent présider à leur incorporation à la terre.

(*Abstract*)

SOME NEW COMPOUNDS OF THE CHOLINE TYPE

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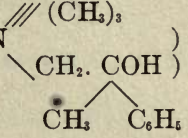
This paper reviews briefly the substance of parts of a preliminary paper previously published under the same title and reports upon methods and procedure involved in the preparation of the following new compounds and upon some of their properties: the acetyl-, pheyl-acetyl-, propionyl-, benzoyl-, vareryl-, monobromisocapronyl-, and palmityl derivatives of α-methylcholine chloride ($Cl.N\begin{cases}///(CH_3)_3\\ \backslash CH.CH_2OH\\ \quad\quad|\\ \quad\quad CH_3\end{cases}$) and their platinum and gold salts; the phenylacetyl derivatives of β-methylcholine ("β-homocholine"—$(Cl.N\begin{cases}///(CH_3)_3\\ \backslash CH_2.CHOH\\ \quad\quad\quad/\\ \quad\quad\quad CH_3\end{cases})$ and of "γ-homocholine" chloride ($(Cl.N\begin{cases}///(CH_3)_3\\ \backslash CH_2.CH_2.CH_2OH\end{cases})$) and their platinum and gold salts.

The synthesis of β-dimethylcholine chloride ($Cl.N\begin{cases}///(CH_3)_2\\ \backslash CH_3.COH\\ \quad\quad\quad/\quad\backslash\\ \quad\quad CH_3\quad CH_3\end{cases}$) and of $\beta.\beta$-methylethylcholine chloride ($Cl.N\begin{cases}///(CH_3)_2\\ \backslash CH_2.COH\\ \quad\quad\quad/\quad\backslash\\ \quad\quad CH_3\quad C_2H_5\end{cases}$), also

the preparation of their platinum and gold salts, were described in the preliminary paper. Similar development of β. β-methylphenylcholine chloride (Cl.N$\begin{  \end{$) and its platinum and gold salts is here reported. The work will be continued.

(From the Department of Experimental Therapeutics, University of Chicago. S. A. Matthews, Director.)

THE RELATION OF THE HYPOPHYSIS TO GROWTH AND THE EFFECT OF FEEDING ANTERIOR AND POSTERIOR LOBE

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As observed by the clinician, there are two clinical entities both disturbances of growth, ascribed to disturbed functioning of the hypophysis. One is acromegaly with over growth of bone, the other Fröhlich's Symdrome with delayed development adiposity and genital atrophy. When the former occurs in early life gigantism results, when it first appears after maturity, enlargement of only certain portions of the bony skeleton is observed. The other type, when the disturbance appears in early life, causes delayed skeletal development, with adiposity and failure of sexual development, when it appears after maturity, adiposity and sexual atrophy.

Pierre Marie in 1886 first called specific notice to the relation between acromegaly and the hypophysis, although Carl von Langer in 1872, in an anatomical study of giants, referred to a certain type with enlarged sella turcica. The observations of Marie have been confirmed until at the present time, it is generally conceded that acromegaly is due to a disturbance of the hypophysis, and according to Sternberg 40 per cent. of the pathologic giants have enlargement of the hypophysis. Regarding the exact nature of the disturbance in the hypophysis in acromegaly, there is still considerable difference of opinion. The weight of evidence, however, favors the view that it is due to hypersecretion of the anterior lobe. The pathologic condition most frequently associated with acromegaly is an adenomatous development of the

anterior lobe with increase in the specific secretory cells. In some instances where enlargement of the anterior lobe is lacking, increase in the specific secretory cells may still be demonstrated, and in addition hyperplasia of the pharyngeal hypophysis should be considered. There is reported in the literature, malignant tumors of the hypophysis with acromegaly. Lewis, who has reviewed these cases, believed that in the majority and possibly all of these the tumor was an adenoma. Although, it is too early to state positively that acromegaly is due to hypersecretion from the anterior lobe, it must be admitted that the weight of evidence supports this view.

The condition of underdevelopment, adiposity, and genital atrophy, first described by Frohlich and referred to as the Frohlich syndrome, all admit is associated with disturbances at the base of the brain, more especially tumors, and has been referred to as cerebral adiposity. Whether in all of these cases the hypophysis is involved, either directly or indirectly by either increased intercranial or intercerebral pressure, has not been determined. On account of its position the hypophysis is especially liable to injury from internal hydrocephalus and it is quite possible that any cerebral disturbance causing increased pressure in the ventricle may compress and disturb the hypophysis. There is one case on record, where a bullet wound of the hypophysis, was followed by adiposity (Madelung). Adiposity may be associated with acromegaly, according to Crenzfeld in 1.6% of cases. In five of the recorded cases of adiposis dolorosa with autopsy, the hypophysis has been abnormal (Lyon). Cases are also on record where removal of the hypophyseal tumor is followed by disappearance of the adiposity (Von Eiselberg).

Assuming that adiposity may arise from disturbances of the hypophysis, it is still to be determined what portion of the gland is responsible for these changes. Fisher believes it is due to hyposecretion of the posterior lobe, and in addition to certain theoretical considerations presents some autopsy findings, where as the result of pressure, the posterior lobe was flattened and had undergone brown atrophy. It can be readily conceived, however, that any pressure involving the posterior lobe must also compress the anterior portions. In all of Fisher's evidence there is little

that is actually convincing, that the posterior lobe plays a role. A more tenable view is that hyposecretion of the anterior lobe is responsible for the adiposity. Zollner in a case of this type found a carcinoma of the anterior lobe. In the five cases of adiposis dolorosa, referred to, with hypophyseal involvement, two showed round cell infiltration of the anterior lobe (Price), one with glioma evidently of the posterior lobe (Burr), one marked increase in size of the anterior lobe from connective tissue hyperplasia and alveolar sarcoma (Guillain), and finally Dercum's case with carcinoma involving both anterior and posterior lobes. Although few deductions may be drawn from the above evidence, on the whole it favors disturbance of the anterior lobe. Most convincing, however, is the experimental evidence. In dogs, removal of the posterior lobe is not followed by any serious consequence, the animal recovers and later fails to show any anomalies of growth, Paulesco, Cushing, Ascola. Where a considerable portion of the anterior lobe of a young dog is removed, and the animal recovers from the effect of the operation, he later develops the Frohlich syndrome of delayed development, adiposity and failure of sexual development, Aschner, Cushing, Casselli, Ascola. This evidence is most suggestive and points strongly toward hyposecretion of the anterior lobe as the cause of the adiposity and sexual atrophy. The question may properly be raised whether the adiposity is due directly to the lessened secretion of the anterior lobe or is secondary to the genital atrophy as Tandler and Grosz have shown that castration in dogs leads to increased deposits of fat and this phenomena is observed in women after removal of the ovaries. It might be argued that it would be exceedingly difficult on the basis of hyposecretion of the anterior lobe to explain the occasional presence of adiposity in acromegaly. Such adiposity is very infrequent according to Creutzfeld in 1.7% of cases, while hypoplasia and gential atrophy were noted in 36.4% of the cases. The greater frequency of the sexual disturbance would lead us to suspect that it and the adiposity were due to separate factors. Here, again, the possibility of the adiposity being secondary to the genital atrophy must be considered its inconstant associations being due to the varying degrees of sexual hypoplasia in the various cases. To those, who believe that the adiposity is due to

the hyposecretion of the posterior lobe, its presence in acromegaly is explained by pressure of the enlarged anterior lobe upon the posterior lobe.

Summarizing it may be said that acromegaly is probably due to increased activity of the anterior lobe. Adiposity, if due directly to disturbances of the hypophysis, is most probably due to hyposecretion of the anterior lobe.

Metabolism is acromegaly. The metabolism in acromegaly has now been studied in a dozen or more cases. Retention of nitrogen is very frequent, often reaching considerable amounts. In some of these cases calcium and phosphorous metabolism are unchanged, in others retention of both have been reported. Seven cases from five different observes all show a retention of nitrogen, five of phosphorous, and five of calcium. Oberndörffer has recently reported two cases and reviewed the literature on this subject. He was unable to detect any variation from the normal in his two cases, and questions whether the results of others are really conclusive on account of the great variation in elimination of normal individuals. Before the work on this phase of subject can be accepted, more extensive studies should be carried out.

Metabolism in animals after the administration of hypophysis. Thompson and Johnson fed dogs upon the entire dried gland of horse, calf and sheep hypophysis and found that they lost in weight and excreted an excessive amount of N and P (Ca and Mg. not determined). They also reported more marked results when glands of young animals were used; Malcomb gave dogs 2–3 gms. daily of dried anterior lobe, for a period of five days, a total of 15 gms. the animal showed slight retention of N. and slightly increased output of P_2O_5 and Ca; after giving posterior lobe (10 gm.) slight increased output of P_2O_5 and marked increased output of Ca. When he gave fresh entire glands 25 gms. daily, there was scarcely any change in the Ca or Mg. output. Franchini injected rabbits, intravenously, daily, with an amount of extract equivalent to one entire hypophysis, this was followed by greater elimination of Ca Mg and P_2O_5 in both urine and feces. The loss in P_2O_5 being less marked than that of Ca or Mg. The animals lost in weight and finally died. The X-ray failed to show any change in the bony skeleton. Some of the animals tolerated

the injection well—others showed marked dyspnea vomiting and diarrhea. Franchini conclusion that hyperpituirtrism leads to loss in weight and failure of development is scarcely justified on account of the severe reaction following the intravenous injection. Oswald gave dogs 2–3 gms. daily of dried hypophyseal extract obtained from Merck (portion of gland not specified) and was unable to detect any change in N or P_2O_5 elimination.

Benedict and Homans working with hypophysectomized dogs and determining carbon dioxide production as an index of total metabolism, found it markedly reduced. The results of these various findings are so at variation, that they throw little light on the disturbance of metabolism following administration of dried hypophysis and may be practically omitted as furnishing definite evidence.

Feeding Experiments. Comparatively few satisfactory feeding experiments have been reported. In some, no attempt has been made to separate the anterior from the posterior lobe. Others have administered the extract subcutaneously or intravenously, Cerletti, Franchini, Delille, Caselli. When given in this way it frequently gives rise to such marked constitutional disturbances, as vomiting and diarrhea, with finally intestinal ulceration, that it is impossible to draw any conclusion regarding the actual effect of the hypophyseal extract. After this method of administration, Cerletti and Franchini report loss in weight and delayed bone development. The entire hypophysis was used and the animals treated for only a few days. Caselli injected young dogs and rabbits with the glycerin extract and did not notice any effect on growth. Delille injected extracts of the entire hypophysis into 4 rabbits for a period of 14 months and reported increased deposits of fat.

Only two references have been obtained of feeding experiments, where the animals received preparations of the hypophysis by mouth for a considerable period of time.

Sandri fed rats on hypophysis exclusively for a period of two months, the controls receiving an exclusive meat diet. While this is an unsuitable diet, Sandri reports that the animals thrived. He found that those fed on the anterior lobe showed greater gain in weight than the controls; when we consult the actual figures,

we find that these differences are so slight that they can scarcely be considered as significant. The controls during the two months gained on an average 10 gms., those fed on the posterior lobe 7 gms., those fed on the anterior lobe 12 gms. Variation of this extent may occur in any group of feeding experiments continued over a period of three months.

Schaefer has conducted the most satisfactory feeding experiments, using, however, only the anterior lobe. Four young rats were fed small amounts of the dried anterior lobe, mixed with bread and milk. The controls received powdered testicle or ovary with bread and milk. The amount consumed by each group of animals was accurately determined. The feeding experiment was continued for about three months. At the beginning, the average weight of the group fed on hypophysis was 44.25 gms. and that of the controls exactly the same. At the end of the feeding, the average weight of those fed on hypophysis was 160 gms. and of the controls 131 gms. During the first six weeks of the feeding, there was little difference between the two groups, during the last six weeks those fed on hypophysis made the more rapid gain. These results would appear to be conclusive, but they are not sufficient in number, to eliminate error.

In undertaking this investigation, it was decided to carry through several series of animals with controls. Young white rats were selected. Each rat was placed in an individual cage, ground cracker was pressed into tablets, each of the same weight. It was then determined how much of this food each rat would consume daily. Although there were some individual differences, it was possible to determine with reasonable accuracy, the daily ration. Having determined this point, Cracker tablets of the requisite weight were made, and to each was added a weighed amount of the hypophysis, or in case of the control meat, and each animal received the same amount daily. Occasionally for a few days, a rat might not eat this whole tablet, if so a note was made of this fact. However, the ration was so arranged that with rare exception, it was consumed daily; and no doubt some of the animals would have eaten more, but the fact that they gained in weight and appeared on the whole healthy would indicate that they were properly fed. By this method, each rat

received and consumed the same amount of food daily, containing the same amount of substance to be tested. The animals were weighed each week.

The ox hypophysis were obtained perfectly fresh from Armour and Co. The anterior and posterior lobes were separated; chopped up fine and dried in a blower at a temperature of approximately 100° F. The dried glands were then powdered and a weighed amount added to the powdered cracker and pressed into a tablet. Three series were fed in this way for about three months each; at the end of the time, the rats were killed and X-rays taken to detect any changes in the bony skeleton. The first series consisted of 9 rats. Three received, daily, .2 gm. of dried anterior lobe, three the same amount of posterior lobe and three controls the same amount of meat. The feeding was continued for 79 days. The second series consisted of 8 rats; four received .4 gms. anterior lobe and the other four as controls received the same amount of dried meat. This group were fed for 90 days. The third series of nine young rats were divided into three groups; one group received .3 gm. daily of beef, another the same

TABLE I—*Series I*

Number of animals	Food daily	Average weight at beginning	Average weight at termination	Change in weight	Period of feeding
		gms.	gms.	gms.	
3	Dried Beef .2 gm.	52.2	91.8	38.6	78 days
3	Dried Anterior lobe .2 gm.	54.2	92.3	37.9	78 days
3	Dried Posterior lobe .2 gm.	58.1	102.6	44.5	78 days
	Series II				
4	Dried Beef .4 gm.	58.	95.2	37.2	90 days
4	Dried Anterior lobe .4 gm.	66.6	107.3	40.7	90 days
	Series III				
3	Dried beef .3 gm.	131.6	144.3	12.6	67 days
3	Dried Posterior lobe .3 gm.	115.3	121.5	6.2	67 days
3	Dried Thymus .3 gm.	118.6	135.3	16.7	67 days

amount of posterior lobe, and the other the same amount of dried thymus. This series was kept under observation for 67 days. By repeating the experiment in this manner, it was thought that some sources of error might be eliminated.

It is interesting to note that animals consuming the same amount of food daily and apparently enjoying equally good health, should show such marked variation of gain in weight. The minimum gain in weight of the controls in Series I was 30.6 gms., the maximum 44.4 gms. In series II the minimum gain in weight of the control rats was 32 gms., the maximum 38.5 gms. In series III, where the rats were $\frac{2}{3}$ grown and, therefore, not so suitable for the test in both the control and those fed on posterior, one of the animals lost 5 gms.

As will be seen by the table in the first series, the controls and those fed upon the anterior lobe showed practically the same gain in weight. Those fed upon the posterior, gained on an average of 6 gms. each more than the controls. When we consider the individual animals, one of those fed on the posterior lobe gained less than one of the controls, the other two gained more than the controls and each animal fed on the posterior lobe gained more than those receiving the anterior lobe. In series III, however, the animals receiving the posterior lobe gained less than the controls and much less than those animals receiving thymus. In series I, animals receiving anterior lobe gained slightly less than the controls, while in series II, they gained somewhat more than the controls. The X-ray pictures of all these animals failed to reveal any variations in the bony skeleton.

Only one conclusion can be drawn from these feeding experiments, viz., that at least in this series of tests neither anterior nor posterior lobes had any effect on the weight or growth of the animal. The experiment was conducted in such a manner that serious causes of error were excluded. The amounts administered were sufficient to give results, as it would be equivalent to 230 gms. daily to the average man—on the other hand, it was not sufficiently large to have a deleterious effect, as the animals so fed gained the same in weight as the controls. Doubling the dose of anterior lobe did not modify results. It must be admitted, however, that this does not prove that disturbed secretion of the

hypophysis may not modify growth. In the feeding experiment, the digestive fluids may destroy the active substances responsible for these changes. Again feeding preparations by mouth can scarcely be considered as analagous, to the continuous secretion occuring in actual life.

Summarizing the entire field of the role of the hypophysis in the growth of the individual. In acromegaly where there exists abnormal development of certain portions of the body especially in their bony structures, there is apparently hypersecretion of the anterior lobe. In the Frohlich syndrome of adiposity and failure of sexual development, it is thought by many that there is lessened function of the posterior lobe. Experimental evidence suggests hyposecretion of the anterior lobe. Regarding studies in metabolism in patients with acromegaly, there is again nothing conclusive, and more work must be carried out upon this subject before it can be accepted that there is a lessened katabolism than in the normal individual.

Turning to the results of partial removal of the hypophysis in animals, only one point, having a direct bearing upon this subject, seems to have been determined, viz: that partial removal of the anterior lobe, when performed upon young animals, modifies growth and sexual development in such a manner as to resemble very closely Frohlich syndrome. Removal of the posterior lobe, apparently, has no effect upon growth. This is a distinct contradiction to those who believe lessened function of the posterior lobe is responsible for the Frohlich syndrome. Feeding experiments, on animals, fail to furnish any definite evidence that the administration of either the anterior or posterior lobe has any effect on growth.

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THE INFLUENCE OF THE CHEMICAL CONSTITUTION OF CERTAIN ORGANIC HYDROXYL AND AMINIC DERIVATIVES ON THEIR GERMICIDAL POWER

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The method employed in estimating the germicidal powers of various organic substances was that devised by Martin and Chick (*Journ. of Hygiene*, 1908, 8, No. 5. Nov. p. 654); it consisted in making a comparison of the concentrations of the substance and of pure phenol required to kill an equal number of organisms of the same species in a constant volume of the disinfectant solution (5 cc) during a constant period of time (15 minutes) and at a constant temperature (20° C).

All test-tubes, pipettes and flasks were first sterilized and different amounts of a standard solution of the substance under examination were introduced into a series of test-tubes, and sterilized water was then added so as to make up each volume to 5 cc. so that the tubes contained a constant volume of solutions containing different concentrations of the substances. A series of phenol solutions was similarly prepared and the two sets of tubes were immersed in a thermostat at 20°. When the tubes had attained this temperature five drops were added to the first dilution from a standard capillary pipette (1 drop = 0.02cc) of a 24 hours' culture of the organism, obtained by inoculating 6 cc. of broth with a standard loopful of agar culture.

The inoculation of the disinfectant solutions proceeded at one minute intervals and at the 15th minute two tubes containing 10 cc. of glucose broth were each inoculated with two loopfuls of the contents of the first tube taken out by means of a standard platinum loop (a loopful of broth weighing about 0.004 gram). The other tubes of the reacting solutions were also subcultured at one minute intervals so that in each case the disinfectant acted for 15 minutes.

The subculture tubes were inoculated at 37° for 96 hours and then examined when the presence or absence of growth was referred to the corresponding dilution. The average of the lowest concentration of the substance which killed and the highest concentration which failed to kill was compared with the corresponding mean phenol concentration and the latter divided by the former gave the carbolic acid co-efficient of the substance.

The organisms used throughout the work were *Staphylococcus pyogenes aureus* and *Bacillus typhosus*. The broth was made according to the following recipe: Brand's meat juice 10 cc, salt 5 grams, peptone 10 grams, glucose 10 grams in 1 litre of tap water. The reaction of the broth was kept constant being + 6 to + 7 to phenolphthalein (Eyre's notation).

The important difference between this method of standardizing disinfectants and the Rideal-Walker method was the selection in the former of a constant reacting time for the germicides, namely 15 minutes.

I. Compounds Containing Hydroxyl Groups

1. *The aliphatic alcohols.*

Jalan de la Croix (*Archiv. f. exp. Pathol.* 1881, p. 175) found that a 1 in 21 aqueous solution of ethyl alcohol prevented the growth of bacteria in broth, but 22 p.c. solutions were required to kill them. Stronger solutions of alcohol (83 p.c.) were necessary to kill spores.

Koch (*Mittheil. a. d. K. Gesundh.* 1881, Vol. 1) found that anthrax spores were not killed by immersion for 110 days in absolute alcohol and in its 33 p.c. and 50 p.c. dilutions. A 1 p.c. solution impeded and an 8 p.c. solution completely arrested the development of anthrax spores.

Fowler (*Journal of the Royal Army Medical Corps.* 1907, July, "Some disinfectant values") found that ethyl alcohol possesses a carbolic acid coefficient of only 0.03 when tested on *B. typhosus.*

There is evidence, therefore, that alcohol possesses feeble germicidal properties. Nevertheless it has sometimes been

employed for antiseptic purposes in surgery, but its chief value is as a vehicle for the application of other therapeutic substances.

All the following alcohols, with the exception of amyl alcohol are freely miscible with water. The carbolic acid coefficients of these compounds were determined on *Staphylococcus py. aureus* in the absence of organic matter.

Alcohol	Concentration required to kill in 15 minutes (parts per 1,000)		Carbolic acid coefficients
	Alcohol	Phenol	
Methyl alcohol	350	9.0	0.025
Ethyl alcohol	350	9.5	0.027
Ethyl alcohol (with *B. typhosus*)	325	8.5	0.026
Propyl alcohol	140	9.0	0.064
iso-Propyl alcohol	210	8.5	0.040
n-Butyl alcohol	41	9.5	0.250
Trimethylcarbinol	190	10.5	0.055

iso-Amyl alcohol (a saturated ($\frac{1}{2}$%) solution at 20° failed to kill in 15 minutes).

The germicidal powers of the foregoing alcohols are considerably less than that of phenol so that the action of the latter must be largely determined by its benzene nucleus. The equality in germicidal powers of methyl and ethyl alcohols is an exception to the general tendency for this action to increase as the homologous series is ascended. This abnormality in the case of methyl alcohol corresponds with the anomalies observed in regard to its chemical and physical properties.

A comparison of the results obtained with *n*-butyl alcohol and trimethylcarbinol shows that the primary alcohol is much superior to the isomeric tertiary alcohol in germicidal power.

Normal propyl alcohol also exceeds its isomeride, *iso*propyl alcohol in germicidal power.

It will be noticed that in the foregoing series of alcohols those members having the higher specific gravity, heat of combustion and boiling point have the greater germicidal power. The phe-

nomenon of germicidal action may be determined by those constitutive influences which determine the physical and chemical properties of substances.

2. The Phenols and Their Homologues and Derivatives

Alcohol is sometimes employed for dissolving the phenols so as to obtain them in a form suitable for disinfecting and antiseptic purposes. For example, there are two non-official preparations of thymol containing ethyl alcohol, liquor antisepticus, and liquor thymol. Kronig and Paul (*Zeitsch.* fur. *Hygiene* 1897, 25, 2, p. 1.) showed that alcohol decreased considerably the germicidal action of phenol on anthrax spores and a solution of phenol in absolute alcohol had very little germicidal power. This inhibiting effect is probably due to the greater solubility of phenol in alcohol, which alters the distribution of this germicide between water and the bacterial proteins with the result that the spores absorb considerably less of the phenol.

In practice the destruction of non-sporing pathogenic organisms is generally the object in view, and it is important to know what effect alcohol has on the germicidal action of phenols on such organisms. Non-sporing microorganisms are more sensitive to the germicidal action of alcohol than are spores, and it was accordingly of interest to ascertain how far the contribution of alcohol towards the bactericidal action of the phenols counterbalanced the inhibiting influence of alcohol on the partition coefficient.

In investigating the effect of known percentages of alcohol on the germicidal action of phenol the experiments were carried out in the manner already described, except that the various dilutions of phenol were made up with sufficient alcohol to bring the percentage of this solvent to the desired extent. The large error involved in the bacteriological test obviated any necessity for a correction for the contraction in volume produced by mixing water and alcohol.

The following table gives the concentrations of phenol required to kill a constant number of *B. typhosus* or *Staphylococci* in 15 minutes at 20° in the presence of various concentrations of alcohol.

Disinfectant	Organism	Percentage of Alcohol					
		0	5	10	15	20	30
Phenol in 1000 parts of solvent	*B. typhosus*	8.5 water 8.5 mean of 4 expts.	8.5	7.0 mean of 2 expts.	6.5	4.75 mean of 3 expts.	1.4 mean of 3 expts.
Phenol in 1000 parts of solvent	*Staphylococcus*	9.5		7.5			

The action of the alcohol on the above non-sporing organisms was sufficiently appreciable to overcome within 15 minutes its depreciating effect on the action of the phenol, so that the presence of this solvent led to an apparent increase in the germicidal efficacy of the phenol, a 0.15 p. c. solution of phenol in 30 p.c. alcohol doing the same amount of disinfection as a 0.85 per cent. aqueous solution. The changes produced by alcohol on the germicidal efficiency of resorcinol and thymol were next investigated.

ORGANISM. *B. Typhosus*

Disinfectant	In Water		In 30 p.c. Alcohol	
	Concentration Killing in 15 mins. at 20°	Carbolic Acid Coefficient	Concentration Killing in 15 mins. at 20°	Carbolic Acid Coefficient
Phenol in 1000 parts solvent	8.5	1	1.1	7.7
Resorcinol in 1000 parts solvent	28.0	0.3	5.0	0.22
Phenol in 1000 parts solvent	7.5	1	1.3	5.6
Thymol in 1000 parts solvent	0.325	23	0.18	7.2

Although 30 p.c. alcohol increases considerably the apparent germicidal effect of resorcinol and thymol on *B. typhosus*, yet as the increase is less marked than in the case of phenol, the car-

bolic acid coefficients of these two substances were reduced by the presence of alcohol of this concentration. Although the use of alcohol cannot be recommended for solutions of phenols required to destroy pathogenic sporing organisms yet this solvent may be employed with advantage in the disinfection of non-sporing organisms, particularly as the phenols are less caustic in alcoholic than in aqueous solution.

As alcohol affects the germicidal powers of different substances to a varying extent, different conclusions may be reached with regard to their efficacy, depending on the medium employed in the process of disinfection, and these irregular variations should be borne in mind in considering the possible relationship between chemical constitution and germicidal power.

Influence of Orientation on the Germicidal Action of the Dihydroxybenzenes

Organism—*B. Typhosus.* Temperature 20°

Dihydroxybenzene	Concentration of substance required to kill in 15 minutes $x/1000$	Concentration of phenol required to kill in 15 minutes $x/1000$	Carbolic Acid Coefficient
Resorcinol	26.0	7.50	0.29
Catechol	17.0	8.25	0.48
Quinol	7.5	8.50	1.1

The minimum effect is produced by the meta-isomeride and the maximum by the para-compound, the ortho-derivative giving an intermediate value of the carbolic coefficient. It is of interest to note that the two isomerides containing hydroxyl groups in sympathetic positions (ortho and para) exert a greater germicidal action than resorcinol in which these groups are in the apathetic meta-position with respect to each other.

The Nitrophenols

The only nitrophenols which appear to have been previously employed as germicides are picric acid (2:4:6—trinitrophenol) and the potassium salt of dinitro-*o*-cresol [$C_6H_2(No_2)_2(CH_3)$ OK.]

Jalan de la Croix (*Arch. f. expt. Pathol.* Jan. 27, 1881) found that 1 in 1000 aqueous solutions of picric acid killed bacteria in infusions of egg-white. Cheron (*J. de Therapeut, Gubler*, 1880, p. 121) used a saturated solution of this compound for purposes of disinfection in hospitals. Koch (*l.c.*) found that 1 in 10,000 dilutions of picric acid impeded the development of anthrax spores, but that 1 in 20,000 dilutions were not sufficient to arrest growth.

Potassium diunitro-*o*-cresoxide has been employed chiefly as an insecticide and a fungicide.

The nitrated derivatives of phenol are not very soluble in cold water but solution was facilitated by gentle warming.

ORGANISM. *Staphylococcus py. aur.* Temperature 20°

Substance	Concentration of substance killing in 15 minutes $x/1000$	Concentration of phenol killing in 15 minutes $x/1000$	Carbolic acid coefficient
p-Nitrophenol	4.6	10.5	2.3
Potassium *p*-nitrophenoxide	20.0	10.5	0.52
m-Nitrophenol	2.7	9.5	3.5
Picric acid	1.4	10.5	7.5
Picric acid with *B. Typhosus*	1.0	8.5	8.5

Potassium *p*.nitrophenoxide contained 2 H_2O of crystallization, and allowing for this the carbolic acid coefficient of dry salt, $No_2 . C_6 H_4 . O K = 0.52 \times {}^{213}\!/_{177} = 0.62$.

The successive introduction of nitro-groups into the phenol molecule produces a progressive increase in the germicidal power of the substance but the practical application of these nitro-compounds is hindered by their poisonous and staining properties. In this series the meta-isomeride is a more powerful germicide than the para-compound. The alkali salt is far less efficacious than the free nitro-derivative, this influence of salt formation will be plainly noticeable in the following series of aromatic hydroxycarboxylic acids.

The following table gives a comparison of the carbolic acid coefficients of the nitrophenols and cresols, the organism employed being *Staphylococcus*.

Nitrophenols	Coefficient	Cresols	Coefficient
1 : 2	—	1 : 2	2.1
1 : 3	3.5	1 : 3	2.0
1 : 4	2.3	1 : 4	2.4

Although *p*-cresol and *p*-nitrophenol have practically the same bactericidal power, *m*-nitrophenol is considerably more active than *m*-cresol.

Saturated solutions of *o*-nitrophenol (0.3 p.c.) and of 2 : 4-dinitrophenol (0.1 p.c.) failed under the prescribed experimental conditions to kill *Staphylococcus py. aur.* in 15 minutes.

COUMARIN, THE COUMARIC ACIDS AND THEIR ALKALI SALTS

The three coumaric acids (hydroxycinnamic acids) combine in their molecular structure the chemical constitutions of cinnamic and salicylic acids and have accordingly been suggested as substitutes for the latter acids in the therapeutic application of these substances. (*British Medical Journal*, 1905, *i.* 1143). The acids have been employed for this purpose in the form of their sodium salts, which are freely soluble in water. The salt of the ortho-acid appeared to be more physiologically active than that of the para-acid whereas the salt of the meta-acid, exerted a more powerful action than either of these substances.

Coumarin is so sparingly soluble in cold water that it was for the purpose of the test, converted into its soluble sodium salt; in these circumstances it dissolves in aqueous sodium hydroxide, forming sodium coumarinate, the *cis*-isomeride of sodium *o*-coumarate which has the *trans*-configuration.

ORGANISM: *B. Typhosus* (24 *hours' culture*)

Substance	Concentration of substance killing in 10 minutes	Concentration of phenol killing in 10 minutes	Carbolic acid coefficient
Sodium *o*-coumarate	1 in 10	1 in 105	0.095
Sodium *o*-coumarinate	1 in 10 failed to kill in 10 minutes		
Sodium *p*-coumarate	1 in 10 failed to kill in 10 minutes		

The results show that the germicidal action of these sodium salts is very feeble, the ortho-coumarate being the most active. This feeble action of soluble alkali derivatives of aromatic phenolic compounds is noticeable in the foregoing case of potassium *p*-nitrophenoxide.

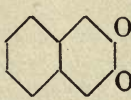
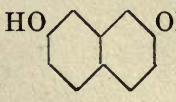
o-Coumaric acid and coumarin are freely soluble in 30 p.c. alcohol and their germicidal action was compared with that of phenol in the same medium.

Substance	Organism	Concentration of substance required to kill in 10 minutes	Concentration of phenol required to kill in 10 minutes	Carbolic acid co-efficient
o-Coumaric acid	*B. coli.*	1 in 500	1 in 620	0.80
o-Coumaric acid	*B. typhosus.*	1 in 500	1 in 680	0.73
Coumarin	*B. typhosus.*	1 in 550	1 in 620	0.56

The Dihydroxynaphthalenes

Although B-naphthol and certain of its sulphonic acids have found employment as germicides the effect of the dihydric naphthols on pathogenic organisms has not hitherto been examined.

1 : 5-Dihydroxynaphthalene is so sparingly soluble in water that its action could not be ascertained in aqueous solution. Two of the 3 isomerides having both their hydroxyl groups in B-positions, namely 2 : 3-dihydroxynaphthalene (m.p. 161°) and 2 : 7-dihydroxynaphthalene (m.p. 190°) were taken for the test, these compounds being sufficiently soluble in cold water, and, as in the foregoing experiments, the carbolic acid coefficient was determined in the absence of added organic matter, *B. typhosus* being taken as the test organism.

Dihydroxy-naphthalene	Concentration of the substance killing in 15 minutes	Concentration of phenol killing in 15 minutes	Carbolic acid coefficient
	1.9 in 1000	8.5 in 1000	4.4
	2.8 in 1000	8.0 in 1000	2.8

The isomeride containing the hydroxyl groups in contiguous positions is the more active, and both compounds are greatly superior to phenol in bactericidal power.

The dihydroxynaphthalenes have not hitherto been suggested for germicidal purposes, and although the cost of preparing the 2 : 3-isomeride would militate against its employment in this direction yet the 2 : 7-isomeride has been prepared economically on a manufacturing scale as an intermediate product in the formation of organic coloring matters. Naphthalene is sulphonated with 5-6 parts of concentrated sulphuric acid for 4 hours at 140°; the resulting naphthalenedisulphonic acid is converted successively into its calcium and sodium salts. The latter is fused with 2 parts of sodium hydroxide and 0.5 part of water, the melt is acidified with dilute sulphuric acid, and, after expelling the sulphur dioxide by means of wet steam, the solution is cooled when 2 : 7-dihydroxynaphthalene separates in almost colorless crystals and is purified by further crystallization from hot water.

It will be noticed that the two dihydroxynaphthalenes examined above greatly exceed the three dihydroxybenzenes in their germicidal action.

II. The Organic Amines

1. *Aliphatic Amines*

Koch (*l.c.*) found that 5 p.c. solutions of trimethylamine in water did not kill anthrax spores in 12 days.

The mixture of aliphatic amines from the interaction of herring-brine and lime has been used for the sterilization of sewage, for which purpose Klein stated that it was very efficient. It consists largely of trimethylamine and under the name of " Aminol " has been introduced as a general disinfectant.

The aliphatic amines used were all freely soluble in cold water. Their germicidal powers determined with *B. Typhosus* are given below.

Amine	Concentration of amine killing in 15 minutes	Concentration of phenol killing in 15 minutes	Carbolic coefficient
Ethylamine	7.09 in 1000	9 in 1000	1.27
Ethylene-diamine	Between 2 p.c. and 30 p.c.	8.5 in 1000	Between.03&.4
iso-Amylamine	3 in 1000	8.5 in 1000	2.8
n-Heptylamine	0.35 in 1000	8.5 in 1000	24.3

The aliphatic amines therefore possess considerable germicidal power which increases with the size of the alkyl group in the amine molecule and which, when *n*-heptylamine is reached, attains a very high value.

The apparently high germicidal power was found *not* to be due to the inhibitory effect of the traces of amine carried over into the sub-culture tubes, as when the contents of the broth-tubes were sub-cultured into a second series of tubes in which the concentration of the amine did not exceed in any experiment .00000025 p.c., the carbolic coefficient was not affected.

Ethylenediamine is much feebler than ethylamine in germicidal power.

The germicidal powers of the fatty amines are compared in the following table with those of the corresponding alcohols:

	ORGANISM. *B. Typhosus*		
Amine	Coefficient	Alcohol	Coefficient
Ethylamine	1.27	Ethyl alcohol	0.026
iso-Amylamine	2.80	*iso*-Amyl alcohol	Under 1.7

The aliphatic amines are therefore considerably superior in germicidal power to the corresponding alcohols.

2. *Aromatic Amines*

Angus Smith (*Disinfectants*, Edinburgh, 1869) examined the germicidal power of aniline and regarded it as a disinfectant of moderate efficiency.

Fischer (*Mittheil a.d. K. Gesundt.* Vol. II) has found that a solution of aniline in water disinfected tubercular sputa in 24 hours.

Many of the aniline dyes have been shown to possess inhibitory and germicidal powers. Stilling (*Lancet*, 1890, Vol. XI. p. 965) showed that dilutions of 1 in 500 to 1 in 1000 of the methyl-violets prevented the growth of moulds on bread and 2 in 1000 dilutions prevented the souring of milk.

Prioux (*Internat. J. of Microscopy, and Nat. Science*, Vol. III, part 18) showed that 1 in 500 to 1 in 2000 dilutions of the methyl violets arrested the growths of *B. typhosus* and *B. coli*.

Fowler (*l.c.*) determined the germicidal power of methylene-blue on *B. typhosus* and found that it possessed a carbolic acid coefficient of 1.5.

Pyridine has been used mixed with oil of peppermint in the treatment of diphtheria and the injection of its aqueous solutions has been beneficial in gonorrhoea (Helbeig, *Mod. Mat. Med.* p. 65). Blyth showed that *Staphylococcus py. aur.* was killed by 1 p.c. solutions of pyridine and its homologues derived from bone-oil.

May found that magenta base (consisting largely of rosaniline) exceeds phenol in germicidal power and is moreover less toxic and more readily diffusible (*J. Amer. Medical Association*, 1912, 8 (16) April 20th).

With the exception of pyridine, the aromatic amines used were not freely soluble in water but solution was accelerated by gentle warming.

ORGANISM. *B. Typhosus*

Substance	Concentration of substance killing in 15 minutes	Concentration of phenol killing in 15 minutes	Carbolic Acid Coefficient
Aniline	15 in 1000	8.5 in 1000	0.57
o-Toluidine	7.5 in 1000	7.5 in 1000	1.00
m-Toluidine	6.5 in 1000	8.5 in 1000	1.30
p-Toluidine	6.0 in 1000	7.5 in 1000	1.25
Pyridine	48 in 1000	8.5 in 1000	0.18
ac-Tetrahydro-*B*-naphthylamine	1.6 in 1000	8.5 in 1000	5.3
o-Phenylenediamine	a saturated solution (2 p.c.) failed to kill in 15 minutes		under 0.42
m-Phenylenediamine	a 4 p.c. solution failed to kill in 15 minutes		under 0.2
p-Phenylenediamine	a saturated solution (3 p.c.) failed to kill in 15 minutes		under 0.3
Tolylene-1:4-diamine	a 4 p.c. solution failed to kill in 15 minutes		under 0.2

The germicidal power of aniline is therefore considerably less than that of phenol. The toluidines exceed aniline in germicidal power, indicating that the introduction of a methyl group into the benzene nucleus of aniline increases bactericidal action. The relative positions of the amine—and methyl—groups has some effect on germicidal power, which is greatest when the groups are in the meta- or para-position to one another.

Of all the monacidic amines examined, whether aliphatic or aromatic, pyridine is the feeblest in germicidal action.

The results obtained with aniline and phenylenediamines and with the toluidines and tolylene—2.4-diamine show that the entrance of a second amino-group into the benzene nuclei of aniline and the toluidines leads to considerable decrease in germicidal power. The aliphatic diamine, ethylene-diamine, is also weaker than ethylamine in bactericidal power.

In the following table the germicidal efficiencies of the toluidines and cresols are compared.

ORGANISM. *B. Typhosus*

Toluidines	Carbolic Coefficient	Cresols	Carbolic coefficient
Ortho.	1.00	Ortho.	2.6
Meta.	1.30	Meta.	2.6
Para.	1.25	Para.	2.6

The cresols are therefore consistently superior to the toluidines in germicidal power.

In the following table the germicidal powers of the dihydroxybenzenes and diaminobenzenes are compared.

ORGANISM. *B. Typhosus*

Amines	Coefficient	Phenols	Coefficient
o-phenylenediamine	Under 0.42	Catechol (1 : 2)	0.48
m-phenylenediamine	Under 0.2	Resorcinol(1 : 3)	0.29
p-phenylenediamine	Under 0.3	Quinol (1 : 4)	1.1

The dihydroxybenzenes are therefore superior to the diaminobenzenes in germicidal power.

The superiority in germicidal efficiency of phenol to aniline, of the cresols to the toluidines and of the dihydroxybenzenes to the diaminobenzenes indicates that the substitution of the amino-group for the hydroxy-group in the benzene nucleus is accompanied by a decrease in germicidal power. In the aliphatic series, on the other hand, the substitution of an amino-group for the hydroxy-group leads to a great rise in germicidal efficiency.

The germicidal powers of the aliphatic and aromatic amines are compared below.

ORGANISM. *B. Typhosus*

Amine	Carbolic Coefficient
Ethylamine	1.27
*iso*Amylamine	2.8
n-Heptylamine	24.3
ac-Tetrahydro-*β*.naphthylamine	5.3
Aniline	0.57
o-, *m*-, & *b*-Toluidines	1.00; 1.30; 1.25
Pyridine	0.18

It is seen that ethylamine exceeds aniline and is approximately equivalent to the toluidines in germicidal power whilst *iso*amylamine and *n*-heptylamine surpass very considerably the aromatic amines in efficiency. High germicidal power in the amines thus seems to be produced not by the presence of groups of an acidic nature such as phenyl and tolyl but by the presence of alkyl groups on which the strong basic properties of the aliphatic amines largely depend.

It is possible that the fatty amines owe their high germicidal efficiency in aqueous solution partially to the presence of hydroxyl ions liberated through ionisation of the alkylammonium hydroxides which are formed by the combination of the amines with water—

$$C_2H_5 \cdot NH_2 + H_2O = C_2H_5 \cdot NH_3 \cdot OH$$

$$C_2H_5 \cdot NH_3 \cdot OH = C_2H_5 \cdot NH_3^+ + OH^-$$

From this point of view, however, the feeble germicidal action of the strong base, ethylenediamine is certainly exceptional.

ac.—Tetrahydro-*β*-naphthylamine has a high carbolic acid coefficient which is noteworthy in connection with the chemical relationships of this amine. Its amino-group is attached to a fully hydrogenized ring; the substance is a strong base, non-diazotisable, absorbing carbon dioxide from the air and soluble in water. The high germicidal power of the base is also of interest

when considered in conjunction with the powerful fever-inducing action of this substance on the higher animals. The replacement of hydrogen in ammonia by radicles of an acidic nature such as phenyl or tolyl, gives rise to substances of feeble germicidal action. Other acidic groups such as succinyl have a similar effect. A 2 p.c. solution of succinimide failed to kill *B. typhosus* in 15 minutes, thus indicating a carbolic acid coefficient of less than 0.4.

The Effect of Age on the Germicidal Power of Aromatic Amines

Most of the aromatic amines gradually become highly colored on exposure and the following comparison has been made of the germicidal powers of aniline and *o*-toluidine in the colored and colorless (redistilled) condition.

Organism. *B. Typhosus*

Amine	Carbolic coefficient	
	Colorless	Colored
Aniline	0.57	0.69
o-Toluidine	1.00	1.20

The effect of exposure is therefore to increase slightly the germicidal powers of these amines.

This fact is of interest in relation to the work of Thalhimer and Palmer (*Journal of Infectious diseases*, Vol. IX, 1911, p. 172) who have recently shown that phenol which had become colored either by age or by exposure to sunlight also possesses a higher germicidal power than fresh colorless phenol.

The authors desire to express their thanks to Dr. C. J. Martin, F.R.S., for laboratory facilities given at the Lister Institute and to the Government Grant Committee of the Royal Society for a Grant which has partly defrayed the cost of the chemicals employed in this investigation.

DOSAGE ET MOYEN DE CARACTERISER DE PETITES QUANTITES D'ALCOOL METHYLIQUE DANS LE SANG ET LES TISSUS

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L'alcool méthylique qui ne présentait jusqu'içi au point de vue pharmacologique qu'un intérêt des plus restreints se trouve aujourd'hui à l'ordre du jour du fait de la récente " épidémie de Berlin (Janvier 1912)."

Sans entrer dans le détail d'expériences que je poursuis en collaboration avec M. Placet sur la toxicité comparée de l'alcool mèthylique et de l'alcool éthylique et sur l'élimination de ces deux alcools, j'indiquerai içi brièvement comment il est possible de doser et de caractériser de petites quantités d'alcool méthylique dans le sang et les tissus.

I° Dosage dans le sang et les tissus. Le sang ou les tissus sont additionnés de 6 à 8 fois leur poids d'une solution saturée d'acide picrique, les tissus coupés et réduits en menus morceaux au sein de la dissolution picrique. On distille dans l'appareil de Schloesing-Aubin. En raison de la présence de l'acide picrique il n'y a pas production de mousse gênante et lorsqu'en a recueilli le $\frac{1}{5}$ de volume total mis à distiller l'alcool se trouve entièrement réuni dans le distillat. Pour l'y doser il suffit d'employer la méthode de dosage par le bichromate de potasse que j'ai publiée il y a seize années, et dont maints auteurs ont reconnu la simplicité et l'exactitude. Elle s'applique en effet parfaitement au dosage de l'alcool méthylique. En employant une solution de bichromate à 19gr. par litre cette solution est telle que 1cc. corresponde à 5cc. d'une solution à 0,5 pour 1000 d'alcool méthylique.

II° Procédé pour caractériser l'alcool méthylique. Dans le distillat qui provient de la distillation du sang ou des tissus, on effectue en vase clos l'oxydation de l'alcool méthylique que l'on veut caractériser par le bichromate de potasse et on recueille et

dose l'acide carbonique produit. Dans ces conditions on possède tous les éléments pour déterminer avec exactitude le rapport $\frac{CO_2}{O_2}$: CO_2 étant mesuré comme il vient d'être dit, O_2 se déduisant avec la plus grande facilité de la quantité de bichromate employé pour arriver au terme de l'oxydation. Or le rapport $\frac{CO_2}{O_2}$ est spécifique, il est égal à 0,915 pour l'alcool méthylique. Si le chiffre obtenu expérimentalement pour la détermination du rapport $\frac{CO_2}{O_2}$ coïncide avec 0,915 aux erreurs d'expérience près, on peut affirmer que l'on se trouve bien en présence d'alcool méthylique et de cet alcool seul.

IL CALCIO E IL MAGNESIO DEL CERVELLO IN DIVERSE CONDIZIONI FISIOLOGICHE E FARMACOLOGICHE

PROF. IVO NOVI

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Sommario.

1 = Il Calcio nel cervello del cane oscilla da gr. 0,0143 a 0,031; il Magnesio da 0,0143 a 0,0167 per cento di sostanza fresca.

2 = L'età ha una grande influenza sul contenuto di Calcio nel cervello. Nei cani la quantità massima si ha nel feto e nel neonato, la minima prima del divezzamento e nell'età avanzata si ritorna alla quantità inziale. Cosi avviene nell'uomo. Nelle cavie invece il Calcio è in quantità minima nei feti, si raddoppia quasi pochi giorni dopo la nascita, continua a crescere per un mese e si mantiene costante fino all'età adulta per accrescer si infine nella vecchiaia, nella quale diviene anche il decuplo.

3 = Introduzioni di NaCl nello stomaco, sotto cute, nella ve ne, nelle carotidi in soluzioni isotoniche ed ipertoniche sottraggono fino al 50% del Calcio al cervello.

4 = Il Magnesio si mantiene sempre costante nel cervello in tutte le età e in tutte le condizioni sperimentali accennate.

5 = Consiglio le cure clorurate nell'arteriosclerosi prima delle lesioni renali, e le diete declorurate nell'osteo malacia e rachitismo.

LA IMPORTANZA FISIOLOGICA DEL MANGANESE NELL'ORGANISMO ANIMALE

Prof. Guido M. Piccinini

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Sommario

1. Il Mn somministrato agli animali, produce non solamente un aumento del Ferro nel sangue, come gli altri agenti ematogeni, ma anche un aumento del Ferro della riserva minerale (fegato e *mil* za). Tra Ferro e Manganese, nei rapporti dell'assimilazione del Ferro, esiste la legge del minimo.

2. Il Mn colloidale produce un aumento dell'ossigeno mobile del sangue e ve lo mantiene per lungo tempo.

3. Il Mn colloidale attenua la virulenza della tossina difterica.

4. Il Mn non deve più essere considerato come un componente accidentale dell'organismo, ma bensi come un elemento costante del corpo animale perchè dotato di due azioni importantissime: la prima; come elemento costitutivo cellulare, la seconda, come elemento *at* tivatore delle ossidazioni.

5. Forse esistono nel corpo umano delle ossidasi manganiche, cioè dei fermenti solubili con lo scheletro metallico dato dal Manganese.

6. Nelle cure ricostituenti dovrebbe essere sempre regola fi siologica la somministrazione associata di Fe e di Mn.

(*Abstract*)

THE ENZYME ACTIVITIES INVOLVED IN CERTAIN PLANT DISEASES

By Howard S. Reed

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The present work deals with the processes involved in the decay of apples by certain fungi, principally the bitter rot of apples, caused by *Glomerella rufomaculans.*

Previous work has shown that several fungi and phytopathogenic bacteria produce cytolytic enzymes which break down the cell walls of their host plants and bring disorganization of the tissues. In some cases a thermostable toxin has been found. In the case studied by de Bary this substance was shown to be oxalic acid.

Glomerella rufomaculans is a fungus which causes a characteristic decay of apples. It causes the pulp to turn brown and lose its tissue organization. After several weeks the tissues shrivel, becoming changed into a hard persistent mass which may resist further disorganizing agents for some time.

The author has shown that when decayed apple pulp is mashed and extracted with water under aseptic conditions, enzymes may be demonstrated in the extract. Oxidizing and reducing exzymes have been shown to exist in such extracts. By the addition of an excess of alcohol to the extracts it was possible to obtain a precipitate containing amylase, invertase, erepsin, and amidase.

More active enzymes were obtained by cultivating the fungus upon sterile nutrient solutions and making an acetone-ether preparation from the mycelium thus obtained.

Amylase was formed by the fungus when cultivated upon any solution, but its production was stronger when starch was the only carbohydrate furnished for the nutrition of the fungus.

The extracellular amylase was weaker than the intracellular form. A small amount of acid favors the action of the amylase, while alkali slightly retarded. Invertase was present in all preparations both as an extracellular and as an intracellular emzyme. Cytase is probably not abundant in the apples affected with bitter rot but it was formed when the fungus was cultivated upon celluose. Inulase was present but weak. Zymase was not present.

In every case an intracellular enulsin was formed which acted upon arbutin, amygdalin, and salicin. No extracellular emulsin was found.

A lipase capable of hydrolysing ethyl acetate and ethyl butyrate was found. Hydrolysis appeared to be greater in the case of ethyl acetate.

Three enzymes capable of acting upon proteins or their cleavage products were found. Protease was identified by its action upon fibrin and a commercial protein. Erepsin was identified by its ability to form tryptophane from peptone and casein. Amidase was identified by the formation of ammonia from alanin and asparagin.

An enzyme splitting hippuric acid into glycocoll and benzoic acid was demonstrated in the enzyme powder.

The thermal death point of the emulsin lay between 55° and 65° C. The death point of invertase and erepsin lay between 70° and 75° C.

The enzymes here demonstrated are such as have the ability to break down certain important constituents of the tissues upon which the fungus grows.

SUR LA NUTRITION MINÉRALE DU BACILLE TUBERCULEUX

Par B. Sauton

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Les divers milieux proposés pour la culture du bacille tuberculeux contiennent tous de la glycérine et un acide amidé. Ils diffèrent profondément par leur composition minérale: le chlore, le sodium, le calcium, le magnésium, le fer, le mangnèse, le zinc, etc. ne figurent pas dans toutes les formules.

Je me suis proposé de déterminer les éléments utiles pour la culture du bacille de Koch et je résume dans le présent travail les premiers résultats obtenus.

Le bacille tuberculeux est cultivé sur un liquide nutritif constitué de produits très purs. Après 20 jours à l'étuve à 38° on pèse le poids de récolte obtenu comparativement sur le milieu complet et sur le même milieu dépourvu de l'élément, dont on veut étudier l'influence.

J'étudie donc uniquement l'influence des éléments sur le poids de récolte obtenu, en laissant de coté l'étude de leur action sur la virulence du microbe et sur la production de la tuberculine.

Les produits employés sont soumis à plusieurs cristallisations successives. La glycérine est purifiée par distillation sous pression réduite. Les divers constituants du milieu sont dissous dans de l'eau soigneusement distillée. Le liquide, neutralisé par l'ammoniaque, est réparti par portions de 100 cc entre des matras de 250 cc, puis stérilisé à 120°.

La formule du milieu nutritif est la suivante:

Asparagine	4 gr. 0/00	Phosphate de potassium	0.5 0/00
Glycérine	60 0/00	Sulfate de magnésium	0.5 0/00
Acide citrique	2 0/00	Citrate de fer ammoniacal	0.05 0/00

Ce milieu, parfaitement limpide, est ensemencé après neutralisation et stérilisation par une portion de voile provenant d'une

précédente culture agée de 8 jours. Le germe employé est un bacille d'origine bovine (BB, LA, ou LP de l'Institut Pasteur).

Après 20 jours de culture, on stérilise l'autoclave à 120°. On filtre sur filtre caré, on lave, on dessèche, on pèse la récolte.

Le poids de récolte obtenu varie de 0 gr. 9 à 1.25 par cc. de liquide. Dans les mêmes conditions, la récolte sur bouillon glycériné est de 0.6 environ. Elle est de 0.35 environ sur le milieu artificiel de Proskauer et Beck. Ce dernier milieu n'est favorable qu'à la condition de renfermer à l'état d'impureté le fer, qui ne figure pas dans sa composition et qui est des éléments nutritifs importants pour la culture du bacille tuberculeux.

Dans le milieu nutritif, utilisé dans le présent travail, le citrate d'ammoniaque n'intervient que pour empêcher la précipitation des phosphates. L'utilité des autres éléments ressort des résultats suivants:

Poids sec après 20 *jours*

Liquide complet	1.15
sans soufre	0.12
sans phosphore	pas de culture
sans magnésium	0.03
sans potassium	pas de culture
sans fer	0.35

Le potassium ne peut pas être remplacé par le sodium, le lithium, le caesium, ni le rubidium. Avec ce dernier métal, on obtient pourtant un début de culture soit que le rubidium puisse être utilisé comme aliment de misère, soit qu'il agisse par le potassium qui l'accompagne à l'état d'impureté.

De même le fer ne peut pas être remplacé par le mangnèse. Les éléments les plus voisins au point de vue chimique sont donc nettement distincts au point de vue biologique.

Au cours de ces premiers essais, il n'y a jamais eu augmentation du poids de récolte par addition de chlore, de calcium, de mangnèse, ou de zinc au milieu de culture.

Pour étudier l'influence du calcium, on substituait, aux matras de verre si facilement attaquables par les réactifs, des capsules de porcelaine. Le calcium n'était décelable dans aucun des produits employés. L'addition de divers sels de cet élément au milieu de

culture ne s'est jamais traduite par une augmentation du poids de récolte obtenu.

L'absence de zinc dans le milieu nutritif a été constatée d'une part par les réactifs chimiques et, d'autre part en utilisant la sensibilité bien connue de l'A. niger pour cet élément. Dans ce but, le milieu non neutralisé par l'ammoniaque était additionné de sucre, puis divisé en deux portions; dans l'une de ces portions on ajoutait 0.04% de sulfate de zinc. On ensemençait ces liquides par des spores d'A. niger. La différence très notable du poids des récoltes indiquait nettement l'absence de zinc dans le milieu nutritif. L'addition de cet élément au liquide qui en était dépourvu ne s'est jamais traduite par une augmentation du poids de récolte du bacille tuberculeux.

Néanmoins, certains éléments pouvant agir à des doses infinitésimales je ne considère pas comme définitifs les résultats obtenus en ce qui concerne les substances dont une première étude n'a pas démontré l'utilité, et je me propose de continuer ces recherches.

SUBCUTANEOUS ABSORPTION OF THYMOL FROM OILS

By W. H. Schultz and Atherton Seidell

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While determining the toxicity of thymol dissolved in various media it was observed that the number of milligrams necessary to cause death varied greatly with the solvent used. Furthermore there appeared to be a relation between the solubility in a given medium and the rate with which the toxic symptoms developed. A series of experiments was therefore planned with the object of ascertaining why, for instance, thymol is more toxic when dissolved in liquid petrolatum than it is when dissolved in olive oil. The necessary solubility and distribution data for comparison with the results of the present experiments upon the subcutaneous absorption of dissolved thymol are presented in detail in another paper from this laboratory. (Section VIII b. Pharmaceutical Chemistry.)

When properly injected a solution of thymol in oil is retained in pocket-like enclosures, the walls of which for most practical purposes serve as an animal membrane through which the thymol must pass. When pure olive oil or pure liquid petrolatum is injected alone so as to be enclosed in one or several of these pockets it is absorbed very slowly, especially the liquid petrolatum. At the end of 90 hours as much as 80 to 90 per cent. of the olive oil can be recovered and liquid petrolatum itself remains for many days as a mass underneath the skin of mice, for the most part where first injected. If, however, oil containing thymol is injected and subsequently withdrawn and analysed the amount of thymol recovered decreases gradually with the increasing interval between injection and collection. Furthermore the amount of thymol recovered will also vary with the solvent used, being less for petrolatum than for olive oil, hence it seemed likely that the

retention of the thymol by oily solvents is proportional to its solubility in the oil tested. The determination of the thymol in the recovered samples of injected oil was made by steam distillation and titration of the distillates by the recently described bromine-hydrobromic acid methold.[1]

The plan of the experiments was as follows. Four sets of mice, *a*, *b*, *c*, *d*, which had been raised upon the same diet, and under the same conditions, were injected subcutaneously in the dorsal region. At first care was taken to have the individuals of each group weigh the same to within a gram, but later it was found that a variation of several grams did not materially alter the results, the main factors seemed to be the time element, the kind of pocket formed and especially the region in which the oil pocket was located. Each mouse received one cubic centimeter of the oil solution. Each cubic centimeter of solution injected contained for sets *a*, *b*, and *c*, respectively, 20, 40 and 60 milligrams of thymol in olive oil, while set *d* was injected with liquid petrolatum containing 20 milligrams of thymol per cc. of solution.

At varying intervals of time after the injection a mouse was chloroformed, the oil pocket carefully exposed by an incision into the skin and the unabsorbed oil drawn into an all-glass syringe, graduated in tenths of a cubic centimeter. The oil thus obtained was estimated to one-hundredths of a cubic centimeter and the sample carefully transferred with the acid of about one half a cubic centimeter of carbon tetrachloride to a distilling flask arranged for steam distillation, about 200 cc. of water were added and the aqueous solution distilled with a current of steam into three 250 cc. glass stoppered bottles. The first, second and third distillates collected in this way were each titrated separately, thus assuring complete removal of the thymol. The quantities of thymol recovered by this method were in all cases somewhat greater than used in the experiment. This constant error is probably due to small amounts of volatile constituents of the oil which react with the bromine used for the titration in a manner somewhat similar to thymol. Since the quantity of oil was kept constant, a correc-

[1]Seidell, Am. Chem. Jour. **47**, 520, 1912.

tion of the apparent excess of thymol is not necessary and the conclusions drawn from the experiments are not affected by this constant error.

The experimental data as summarized in Tables 1, 2 and 3 have been plotted on cross-section paper and average curves constructed (Fig. 1). The points corresponding to the different sets of mice are shown differently and indicate, for all except the liquid petrolatum experiments, that the individual variations are much greater than the differences between the adjoining curves. In spite of this, however, the general directions of the three olive oil curves probably indicate in a general way the rate of absorption of thymol from this oil.

Analysis of the data lead to the following

TABLE No. I. Absorption of Thymol from Olive Oil. Subcutaneous Injection in Mice

Thymol Solution = 20 Mgs. per 1 cc. Olive Oil. Amount injected = 1 cc.

Mouse No.	Mouse Wt. in Gms.	Time Hrs.	cc. Oil Recovered	Gms. Thymol Found	Gms. Thymol per cc. Recovered Oil	Mg. Thymol Absorbed
Blank	— 1 cc. Olive Oil Sol. of Thymol				0.0261	——
Blank	— 1 cc. Olive Oil Sol. of Thymol				0.0242	——
Blank	— 1 cc. Olive Oil Sol. of Thymol				0.0247	——
Blank	—— 1 cc. Pure Olive Oil				0.0020	——
	1 cc. Pure Olive Oil 18 hrs.		0.78	0.0026	0.0033	——
96	23	$\frac{1}{4}$	0.74	0.0176	0.0238	1.2
94	18	$\frac{1}{2}$	0.89	0.0194	0.0218	3.2
103	24	$1\frac{1}{2}$	0.9	0.0192	0.0213	3.7
93	18	2	0.79	0.0144	0.0183	6.7
98	27	$3\frac{1}{4}$	0.93	0.0178	0.0191	5.9
95	25	4	0.92	0.0180	0.0196	5.4
97	23	5	0.9	0.0178	0.0198	5.2
104	24	6	0.77	0.0111	0.01446	10.5
123	25	$13\frac{1}{4}$	0.5	0.0090	0.0180	7.0
99	18	17	0.76	0.0111	0.0142	10.8
124	32	$19\frac{1}{3}$	0.72	0.0086	0.0119	13.1
105	29	$22\frac{1}{2}$	0.91	0.0156	0.0172	7.8
		24	0.60	0.0036	0.0059	19.1
		46	0.90	0.0080	0.0096	15.4
100	23	47	0.78	0.0047	0.0061	18.9
106	23	$71\frac{1}{2}$	0.74	0.0033	0.0045	20.5

TABLE No. 2. Absorption of Thymol from Olive Oil. Mice—Subcutaneus Injection

Thymol Solution = 40 Mgs. per 1 cc. Olive Oil. Amount injected = 1cc.

Mouse No.	Mouse Wt. in Gms.	Time Hrs.	cc. Oil Recovered	Gm. Thymol Found	Gm. Thymol per cc. Recovered Oil	Mg. Thymol Absorbed
Blank 1 cc. Olive Oil Solution					0.0450	—
134	24	$\frac{1}{2}$	0.92	0.0409	0.0444	0.6
133	25	$\frac{3}{4}$	0.92	0.0391	0.0425	2.5
130	31	1	0.88	0.0347	0.0395	5.5
112	31	1	0.81	0.0307	0.0375	7.5
135	25	2	0.83	0.0368	0.0443	0.7
131	25	2	0.94	0.0385	0.0409	4.1
125	24	$2\frac{1}{2}$	0.84	0.0344	0.0410	4.0
115	31	$4\frac{1}{2}$	0.90	0.0359	0.0400	5.0
127	29	$4\frac{1}{2}$	0.91	0.0325	0.0357	9.3
136	31	6	0.90	0.0290	0.0322	12.8
121	26	12	0.72	0.0245	0.0340	11.0
111	—	$17\frac{1}{4}$	0.80	0.0195	0.0241	20.9
114	21	$20\frac{2}{3}$	0.72	0.0199	0.0276	17.4
126	21	$94\frac{1}{3}$	0.82	0.0065	0.0079	37.1

Thymol Solution = 60 Mgs. per 1 cc. Olive Oil. Amount injected = 1 cc.

Blank 1. cc. Olive Oil Solution					0.0641	—
113	32	$\frac{3}{4}$	0.91	0.0553	0.0607	3.3
109	24	$2\frac{1}{3}$	0.85	0.0459	0.0541	10.0
110	24	5	0.82	0.0438	0.0534	10.6

TABLE No. 3. Absorption of Thymol from Liquid Petrolatum. Mice—Subcutaneous Injection

Thymol Solution = 20 Mgs. per 1 cc. Petrolatum. Amount injected = 1cc.

Mouse No.	Mouse Wt. in Gms.	Time Hrs.	cc. Oil Recovered	Gm. Thymol Found	Gm. Thymol per 1 cc. Recovered Oil	Mgs. Thymol Absorbed
Blank 1 cc. Petrolatum Solution					0.0239	—
117	25	½	0.72	0.0154	0.0215	2.5
118	28	1	0.94	0.0182	0.0194	4.6
119	26	2½	0.905	0.0144	0.0159	8.1
120	26	5	0.91	0.0113	0.0124	11.6
128	30	11½	0.92	0.0069	0.0075	16.5
129	29	12	0.85	0.0051	0.0060	18.0
122	25	24	0.95	0.0043	0.0046	19.4
116	27	40	0.95	0.0016	0.0017	22.3

Conclusions: (1) The rate of absorption is greatest during the first hour following subcutaneous injection. The rate of absorption per unit of time gradually diminishes so that the curve is roughly parabolic. For one reason or another some mice absorb slowly whereas others absorb rapidly. Indeed if a sufficient number of experiments be performed it is possible to plot curves of the rate of absorption, one of which will represent the rapid type and the other the slow type of absorption. This variation is independent of season, of diet or of environment, it seems to be due to an inherent difference in the mouse itself and is probably congenital. It is illustrated by the sets of high and low points plotted in connection with both curves *o* and *x* of Fig. 1. The curves representing the rapid and slow types of absorption each show minor deviations above or below a mean rate of absorption. These deviations, however, are mainly accounted for by the character of the injection pocket, its position underneath the skin and certain other factors of technique. Curves *o*, *x*, and □, therefore, represent the mean values for olive oil containing, respectively, 20, 40 and 60 milligrams of thymol per cubic centimeter of solution.

(2) The rate of absorption of thymol injected subcutaneously is decidedly influenced by the amount of thymol contained in one cubic centimeter of olive oil. When mice are injected with olive oil containing 20 milligrams of thymol per cubic centimeter of solution a curve like *o* Fig. 1 is obtained. If a 40 milligram solution be employed a curve slightly steeper and on a higher level is obtained whereas with a 60 milligram solution the curve is still steeper. The higher doses, however, are so toxic that considerable influence is apt to be exerted by the absorbed drug acting as a general depressant. Not only are the cells of the membranous injection pocket affected by the thymol, but the current of circulating body fluids is greatly retarded so that those mice which very early show marked signs of depression may after a long interval, five to fifteen hours, yield oil that contains considerable thymol, showing that absorption had been retarded because of the various physiological factors mentioned.

Absorption of thymol from liquid petrolatum containing 20 milligrams of thymol per cubic centimeter of solution is of unusual interest. (1) The absence of individual variations from the observed rate of absorption is noteworthy. (2) This mineral oil is very slowly absorbed from underneath the skin of white mice; it is apparently but slightly acted upon by the body fluids or enzymes. (3) Liquid petrolatum dissolves at 37° C. only 0.39 as much thymol as does olive oil at the same temperature. A solution of liquid petrolatum containing 20 milligrams of thymol per cubic centimeter is therefore more nearly saturated with thymol than is olive oil of the same concentration, hence diffusion ought to be more rapid from the liquid petrolatum solution. Actual experiment demonstrates the correctness of this assumption. Within certain limits, therefore, when equal volumes of different inert oils contain equal amounts of thymol the rate of subcutaneous absorption is proportional to the relative saturation of the solvent with thymol.

Conclusions

1. Thymol when dissolved in oil and injected underneath the skin of white mice is absorbed from the oil much more quickly than the oil itself is absorbed, the rate of thymol absorption increasing with the concentration of thymol in a given oil.

2. The rate of absportion from oils varies with the oil used and with the partition coefficient between thymol in oil and water. Within certain limits, when equal volumes of the solution of thymol in different inert oils contain equal amounts of thymol the rate of subcutaneous absorption is proportional to the relative saturation of the solvent with thymol.

3. Thymol is absorbed more rapidly at the beginning of the experiment than it is some hours later. This change in rate is probably due to a diluting of the injected solution, to local action of the thymol, and to the general action that it has upon the cardiac and respiratory apparatus.

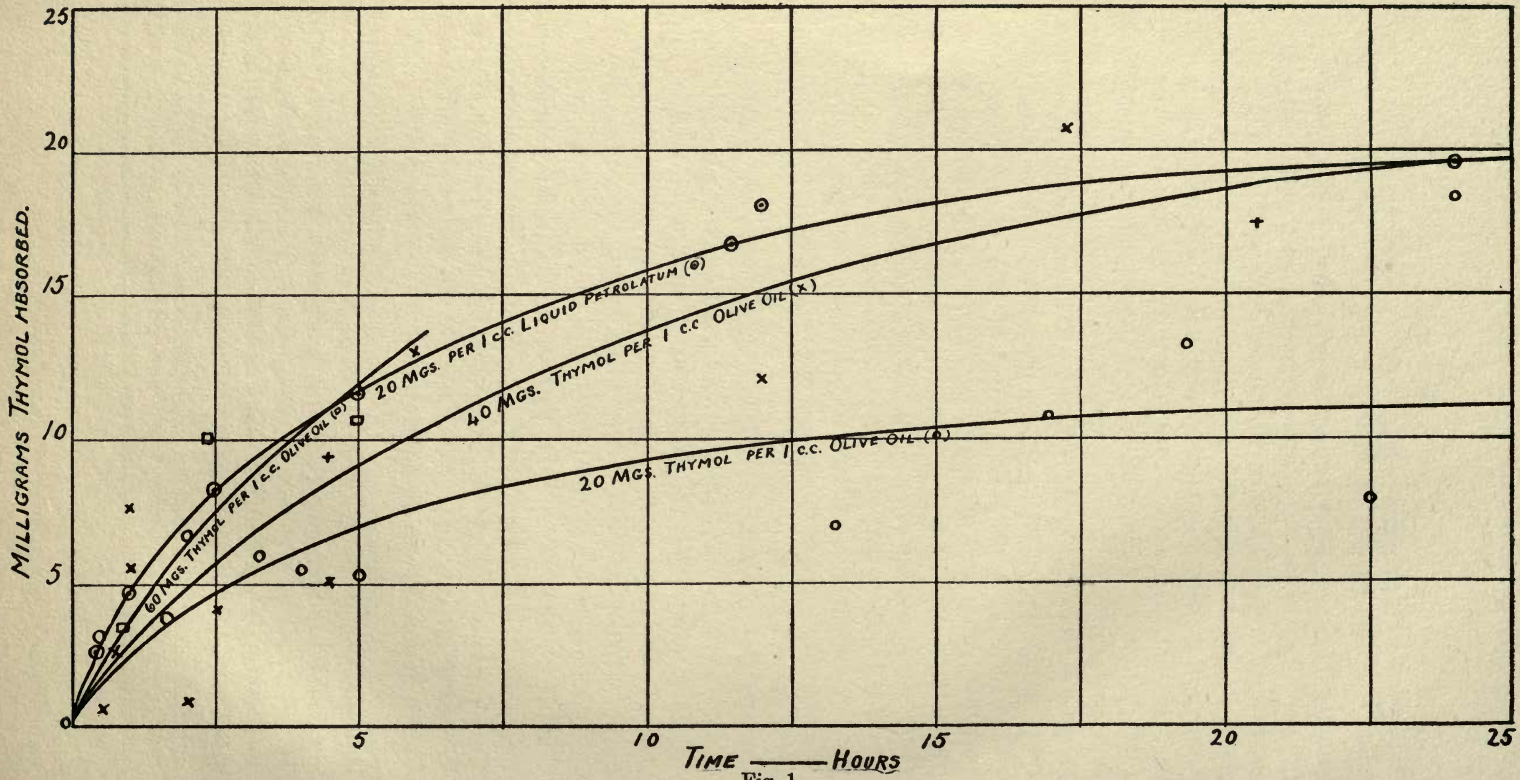
25
20
15
10
5
0
MILLIGRAMS THYMOL ABSORBED.
5
10
15
20
25
TIME ——— HOURS
20 MGS. PER 1 C.C. LIQUID PETROLATUM (⊙)
40 MGS. THYMOL PER 1 C.C OLIVE OIL (x)
60 MGS. THYMOL PER 1 C.C. OLIVEOL (□)
20 MGS. THYMOL PER 1 C.C. OLIVE OIL (○)

Fig. 1.

THE DETERMINATION OF THYMOL IN DOG FECES

By W. H. Schultz and Atherton Seidell

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It has frequently been observed during the examination of feces for hook-worm eggs that samples from patients having received large doses of thymol may show undoubted evidence of the presence of this drug in an unaltered state. Since it has long been known that thymol is eliminated through the urine in combination with glycuronic and other acids,[1] it appeared of interest to ascertain what proportion comes through the alimentary tract unchanged. A satisfactory quantitative method for the determination of thymol having been devised by one of us[2] it was decided to attempt to apply this method to the determination of thymol in dog feces.

On account of the requirements of the method, that the thymol solution be neutral and contain no substance which is acted upon by bromine, it was decided to take advantage of the volatility of thymol with steam in order to separate it from the fecal material. Experiments were therefore made for the purpose of ascertaining the proper conditions for the steam distillation of the samples of feces in order that the least possible amount of interfering substance would be obtained in the distillate. It was found that distillation from an acidified mixture gave an acid distillate and from an alkaline medium a more or less strongly alkaline one. A double steam distillation from first an acid and then an alkaline mixture was then adopted and, as might be expected, gave a practically neutral distillate; blank determinations showed that the alkaline medium which was used, viz. magnesium oxide suspended in water, did not retain an appreciable quantity of thymol. When, however, determinations were made upon mixtures of feces and known amounts of thymol, high results were

[1]Blum, Z. physiol. Chem., **16**, 514-24. 1892.

[2]Seidell, Am. Chem. Jour., **47**, 520. 1912.

invariably obtained. After many experiments it was ascertained that the cause of the trouble was the hydrogen sulphide which passed readily through the second distilling flask containing the magnesium oxide in suspension. In attempting to retain this hydrogen sulphide it was found that the addition of lead nitrate was quite effective, but if used in the first distilling flask containing the acid mixture the thymol was also held back to a greater or less extent. When used in the second distilling flask with the aqueous suspension of magnesium oxide it exerted no influence whatever upon the thymol and completely retained the hydrogen sulphide.

There are, of course, in feces very small amounts of certain phenols and phenol-like compounds, for instance, indol, skatol, etc., which might be expected to yield bromine substitution products very much as thymol. Blank determinations run with feces show, however, that although a certain amount of hydrobromic acid is formed when these distillates are treated with bromine vapor, the necessary correction is small.

The details of the method which our experiments have finally led us to adopt are as follows: The apparatus consists of an ordinary steam generator made from an empty ether can, and two 500 cc. distilling flasks connected in series for steam distillation. The distillate from the second flask passes into a condenser and is received directly into the 250 cc. glass stoppered bottle in which the titration is to be made. Three of these bottles should be provided to collect the first, second and third distillates, each of which should come over in about 20 to 30 minutes and measure about 150 cc. A convenient amount of feces to use is about 20 grams and, as will be seen from the following table, the amount of thymol should be approximately 0.2 gram in order that a suitable amount of the standard thiosulphate be required for the titration. In all of the determinations shown in Table No. 1 the thymol was added in the form of 1.0 per cent. solution made by dissolving it with gentle warming in just a little more than the calculated amount of normal sodium hydroxide to form the sodium salt of thymol and diluting with the necessary amount of water. The feces were in some cases the hard white lumps and in others darker and softer masses. In each case the first flask contained in addition

to the feces and thymol about 100 cc. of water and 3 cc. of phosphoric acid solution, made by diluting the ordinary 85 per cent. H_3PO_4 syrup with an equal volume of water. The second distillation flask in all cases contained approximately five grams of magnesium oxide suspended in about 100 cc. of aqueous 1.0 per cent. $Pb(NO_3)_2$ solution. The distillate was immediately treated with about 1 cc. of CCl_4, and then the bromine vapor was poured in, a little at a time, with alternate shaking and addition of bromine until the mixture retained a distinct red brown color. It was then allowed to stand in a dark place about one-half hour five cc. of CS_2 and 5 cc. of 20 per cent. KI solution were added and the bottle well shaken, standard thiosulphate solution was run in until the pink color of the iodine in the CS_2 layer was just discharged, an additional amount of KI solution was added and if no further liberation of iodine occurred the reading on the burette was taken. Five cc. of 2 per cent. KIO_3 solution were then added and after thorough shaking the titration with thiosulphate was continued until the iodine color was just discharged for the second time. The completion of the reaction may be tested by a further addition of KI and KIO_3 solutions. The difference between the first (which should be from about 5 to 15 cc. 0.1 n thiosulphate) and second reading corresponds to the hydrobromic acid formed by the action of the bromine on the thymol. The calculation is made on the basis of two molecules of HBr per one of thymol; 1 cc. 0.1 n thiosulphate is, therefore, equal to 0.0075056 gram thymol.

TABLE No. 1. Showing the Results of the Determination of Thymol when Mixed with Dog Feces in Various Proportions.

Composition of Sample		Distillates Obtained		Gm. Thymol Recovered	
Gms. Feces	Gm. Thymol	No.	Volume		
—	0.2	1st	150	0.194	
20	0.2	1st	150	0.192	
20	0.05	1st	120	0.051	0.063
		2nd	120	0.012	
		1st	130	0.096	
20	0.1	2nd	110	0.010	0.115
		3rd	150	0.009	
		1st	120	0.343	
20	0.5	2nd	110	0.076	0.444
		3rd	110	0.025	
		1st	110	0.0143	
80	0	2nd	140	0.0089	0.027
		3rd	120	0.0036	
		1st	125	0.153	
50	0.2	2nd	140	0.047	0.224
		3rd	150	0.024	
10	0.2	1st	160	0.169	0.186
		2nd	145	0.017	
5	0.2	1st	90	0.178	0.182
		2nd	120	0.014	
		1st	100	0.311	
	0.40	2nd	110	0.057	0.380
		3rd	100	0.012	
		1st	100	0.380	
—	0.40	2nd	125	0.015	0.399
		3rd	140	0.004	

Although the results shown in Table 1 are not entirely as satisfactory as could be desired, further work upon the improvement of the method was not done since preliminary experiments upon the feces of dogs to which small doses of thymol had been given showed that only insignificant amounts of thymol were present.

PROTOCOLS OF EXPERIMENTS

1. Dog No. 6, Wt. 6.95 kilograms, amount of thymol given = 1.0 gram.

Samples of Feces Time	Amt. Gms.	Apparent Gas. Thymol in sample	Correction per 45 Gms. Feces	Thymol Recovered
Before administration of thymol	56.0	0.028		
5 ⅔ hrs. after administration of thymol	45.0	0.100	—0.022 =	0.078
24 hrs. after administration of thymol	40	0.029	—0.022 =	0.007
				0.085

The correction per 45 gms. feces is obtained from the blank determination made upon the feces obtained before the administration of the thymol. Since the total amount of administered thymol was 1.0 gram, it is apparent that not more than 8.5 per cent. of it came through the alimentary tract unchanged.

2. A second experiment with Dog No. 6 was made four days later. The amount of thymol given was 0.5 gram. The total feces was collected in several portions during the first 25 hours after administration of the thymol and amounted to 49 grams. The apparent thymol recovered from this quantity was 0.046 gram and this figure corrected for the blank determination as shown in the previous experiment is 0.046 - 0.22= 0.024 gram thymol recovered from 0.5 gram administered or approximately 5 per cent.

3. Dog No. 26 was given 0.5 gram thymol at 10 o'clock A. M. but vomited 4 - 5 hours afterwards. The combined vomit was distilled and found to contain approximately 0.05 gram thymol. The administered thymol as corrected for this amount is therefore 0.45 gram.

Samples of feces Time	Amt. Gms.	Apparent Gm. Thymol in sample	Correction per 45 Gm. Feces	Thymol Recovered
3 hrs. after administration of thymol	15	0.012	0.007	0.005
23 hrs. after administration of thymol	44	0.040	0.022	0.018
				0.023

From these results it is seen that about 5 per cent. of the thymol came through the alimentary tract.

Although these experiments are not as numerous as desirable they show conclusively that when small doses of thymol are given only insignificant amounts are eliminated unchanged with the feces.

Preliminary experiments with the *urine* from dogs which had received thymol showed that all of the drug excreted in this manner is in firm combination, probably with glycuronic acid. Such urines were found to yield no appreciable amount of thymol from neutral solution, by steam distillation, but did so when considerable free acid was used in the distilling flask. Quantitative results, however, have so far not been obtained since the distillation method has not been developed to the extent of eliminating certain interfering substances that are distilled with thymol. These experiments are still in progress and it is hoped to improve the technique so that it will be possible to account for nearly all of the ingested thymol.

SUR LA RÉSISTANCE DE LA PEROXYDASE A L'AMMONIAQUE ET SUR SON ACTIVATION PAR CONTACT AVEC L'ALCALI

PAR M. J. WOLFF

Paris

J'ai constaté dans les jeunes pousses d'orge l'existence d'une peroxydase très active et particulièrement résistante à l'action de la chaleur, attendu qu'elle n'est détruite qu'après plusieurs minutes d'ébullition. Comme les autres peroxydases connues, celle des pousses d'orge est détruite presque instantanément par de faibles doses d'acide sulfurique et phosphorique,[1] et résiste, sans être sensiblement affaiblie, à des doses équivalentes de bases alcalines.

J'ai étudié cette action des alcalis et j'ai vu que si des doses un peu massives de soude sont capables de détruire la peroxydase au bout de quelques heures, des quantités équimoléculaires d'ammoniaque n'attaquent l'enzyme qu'avec une extrême lenteur.

EXEMPLE: Je mets en contact d'une part 1 cc. de macération diastasique avec 3 cc. de soude normale; d'autre part 1 cc. de la même macération avec 3 cc. d'ammoniaque normal. Je constate alors qu'au bout de 8 à 10 heures l'enzyme a été complètement détruit par la soude. La peroxydase qui a été laissée en contact avec l'ammoniaque conserve ses propriétés pendant 8 à 10 jours. Au bout de ce temps elle est affaiblie, mais nullement détruite.

Ce fait inattendu m'a suggéré l'idée d'étudier à l'aide d'expériences plus délicates ce qui se passe lorsqu'on laisse en contact la peroxydase avec l'ammoniaque, et de suivre les modifications qui peuvent survenir au cours de cette action.

Pour étudier les différentes phases du phénomène, je me suis servi comme réactif du gayacol en présence d'eau oxygénée et

[1] Il n'est pas indifférent de remarquer que la peroxydase résiste mieux à de faibles doses d'acide phosphorique qu'à de faibles doses d'acide sulfurique.

j'ai toujours exécuté la réaction dans un milieu renfermant un faible excès de phosphate acide de potassium, en prenant comme mesure l'intensité de la coloration produite et la rapidité de son apparition. C'est ainsi que j'ai pu observer un ralentissement considérable de la réaction tout à fait au début de l'expérience, par comparaison avec un témoin sans alcali; puis par des prélèvements opérés d'heure en heure sur le même mélange de peroxydase et d'ammoniaque, j'ai vu l'activité augmenter de plus en plus à mesure que le contact se prolongeait. La marche du phénomène montre que l'activité perdue au début est regagnée, puis considérablement dépassée.

Les diverses phases du phénomène peuvent se résumer ainsi:

1°. Au moment où la peroxydase entre en contact avec l'ammoniaque, il y a une perte considérable de l'activité primitive.

2°. A mesure que le temps de contact s'accroît, l'activité s'accroît.

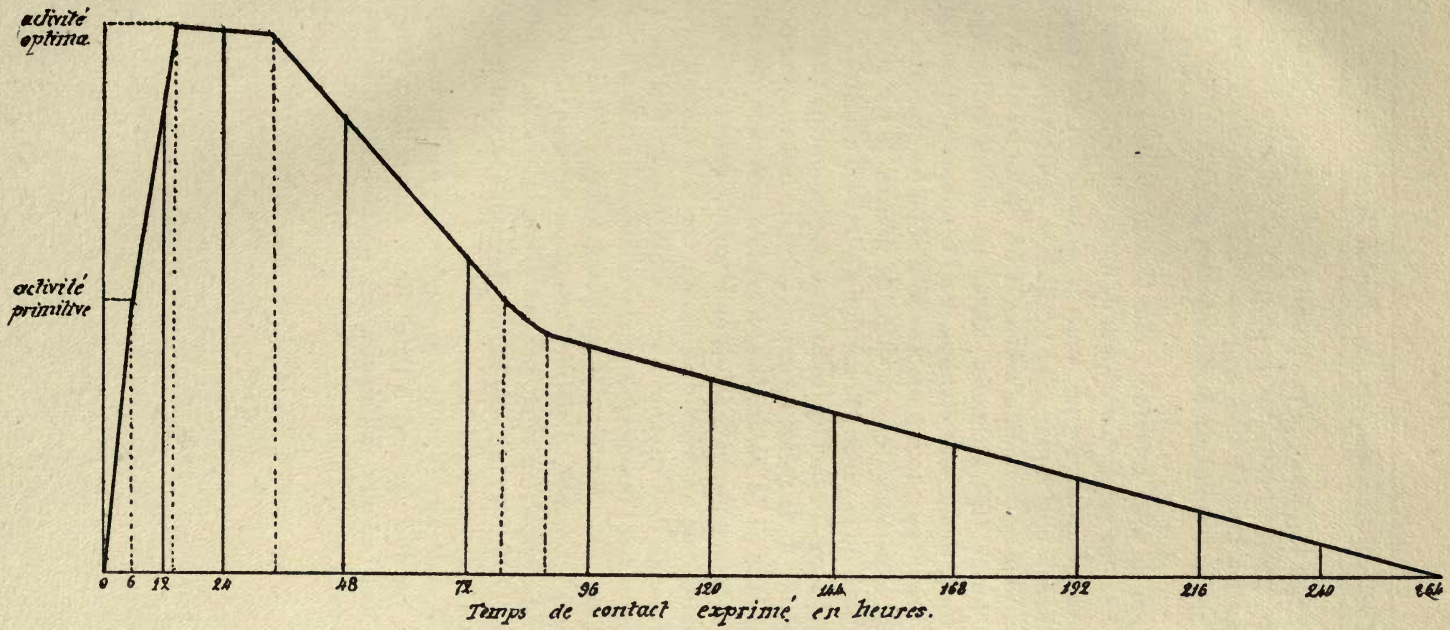
3°. Au bout de 4 à 5 heures, cette activité, a repris sa valeur primitive.

4°. A partir de ce moment, l'intensité de la réaction produite continue à s'accroître et l'activité atteint son maximum vers la 14^{e} heure; cette activité représente environ le double de l'activité primitive de la peroxydase.

5°. L'activité de la peroxydase reste ensuite sensiblement constante pendant quelques heures, puis elle décroît lentement.

6°. Au bout de 11 jours, l'activité est de nouveau très affaiblie, et elle est comparable à ce qu'elle était à son début, au moment du premier contact (en 1).

On peut essayer, au moyen de ces données, de représenter grossièrement le phénomène d'activation par une courbe. Si on adopte pour 0 l'instant précis où l'activation commence, et si l'on porte les temps de contact en abscisses et les activations en ordonnées, ce que l'on peut faire si l'on observe que l'activité maxima est le double de l'activité primitive ou normale, on aura le tracé suivant:



Courbe des vitesses de réaction

On voit par la forme de la courbe que la marche du phénomène se rapproche beaucoup de ce que l'on observe habituellement dans les actions diastasiques. La diminution lente de l'activité est le résultat de la destruction progressive de la peroxydase.

Il est bon de faire remarquer que le contact de 1 cc. de peroxydase avec une solution *décinormale* de soude peut donner lieu à des phénomènes analogues, mais la destruction de l'enzyme étant beaucoup plus rapide avec la solution *décinormale de soude* qu'avec la *solution normale d'ammoniaque*, on ne les observe que sous une forme très atténuée. Avec les acides sulfurique et phosphorique, même très étendus, ces phénomènes d'activation ne se produisent pas; tout au moins je n'ai pu les observer.

Dans les expériences que je viens de décrire, je me suis servi comme réactif du gayacol. Lorsqu'on s'adresse à d'autres réactifs tels que le pyrogallol ou l'hydroquinone, le phénomène ne se passe pas tout à fait de la même façon; en effet, on observe *une activation immédiate de la peroxydase*, lorsqu'en présence de celle-ci et d'un excès de phosphate acide, on introduit dans le milieu une petite quantité de soude ou d'ammoniaque. Un contact plus ou moins prolongé de l'enzyme avec l'ammoniaque n'a pas pour effet d'augmenter l'intensité de la réaction comme cela a lieu dans le cas du gayacol. Je n'ai pu jusqu'ici m'expliquer ces différences. Toutefois, je crois utile d'attirer l'attention sur leur importance. On se rend compte, en effet, par cet exemple, que la substance qui subit l'action de l'enzyme est aussi sensible aux influences du milieu que l'enzyme lui-même.

Enfin, il se dégage de toutes ces expériences que les bases alcalines, soit qu'elles agissent à l'état libre, soit à l'état combiné, sont un des facteurs principaux des phénomènes d'activation analysés dans ce travail.

GENERAL INDEX
TO THE TWENTY-FOUR VOLUMES OF ORIGINAL COMMUNICATIONS

Volume	Section	
1	I.	Analytical Chemistry.
2	II	Inorganic Chemistry.
3	IIIa	Metallurgy and Mining.
4	IIIb	Explosives.
5	IIIc	Silicate Industries.
6	IV	Organic Chemistry.
7	IVa	Coal Tar Colors and Dyestuffs.
8	Va	Industry and Chemistry of Sugar.
9	Vb	India Rubber and other Plastics.
10	Vc	Fuels and Asphalt.
11	Vd	Fats, Fatty Oils and Soaps.
12	Ve	Paints, Drying Oils and Varnishes.
13	VIa	Starch, Cellulose and Paper.
14	VIb	Fermentation.
15	VII	Agricultural Chemistry.
16	VIIIa	Hygiene.
17	VIIIb	Pharmaceutical Chemistry.
18	VIIIc	Bromatology.
19	VIIId	Biochemistry including Pharmacology.
20	IX	Photochemistry.
21	Xa	Electrochemistry.
22	Xb	Physical Chemistry.
23	XIa	Law and Legislation Affecting Chemical Industry.
24	XIb	Political Economy and Conservation of Natural Resources.

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